

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Fluorescent Dyes Introduction**

Fluorescent dyes are routinely used in oligonucleotide-based research as detection labels for primers and probes. Single-dye-labeled oligos are effective as primers for sequencing, AFLP and microsatellite fragment analysis, and single-dye-labeled probes for fluorescent in situ hybridization (FISH) and oligonucleotide ligation assay (OLA) applications. Dual-labeled probes incorporating various matched dye and quencher combinations are often indispensable for quantitative experiments. Fluorescence-based detection offers a safe and sensitive method for both qualitative and quantitative detection of target sequences in vitro and in vivo. The elegant design of the newer probes has led to an exponential increase in the use of molecular probes, furthering new developments. Gene Link offers synthesis of all different forms of molecular probes and knowledgeable technical service in the design of novel probes, including chimerics.



### **Fluorescent Dyes Design Protocols**

### Use of Controls in Fluorescence-based Real-Time Quantitative PCR (RT-qPCR) reactions—Design Considerations

A variety of fluorescence-based systems can be used to analyze samples using RT-qPCR, including sequence-specific nucleic acid probes (ex: 5'-nuclease probes, molecular beacons, FRET hybridization probes, Scorpions) and non-sequence-specific fluorescent intercalating dyes (ex: SYBR Green, Cyto dyes). No matter which system you choose, however, it is critically important that the proper negative and positive controls be run to ensure that your experimental results can be correctly interpreted. The following controls should be part of every RT-qPCR experiment. I. Negative Controls (each run in a separate tube)

- a) a no-reverse-transcriptase control: For this control, the reverse transcripase enzyme is not included in the reverse transcription reaction. Performing this control allows you to determine the amount of genomic DNA contamination in your RNA preparation.
- b) a no-template control: For this control, the cDNA template is not included in the PCR reaction. Performing this control allows you to determine the amount of nucleic acid contamination and/or primer-dimer formation. It is particularly important to do this control if you are using a fluorescent intercalating dye for detection.
- c) a no-amplification control: For this control, the DNA polymerase is not included in the PCR reaction. Performing this control allows you to determine background fluorescence in the PCR reaction. II. <u>Positive Controls</u>
- a) exogenous positive control (run in a separate tube): For this control, external RNA or DNA, containing the target of interest (but NOT one of your experimental samples), is added to the reverse transcription or PCR reaction in place of an experimental sample. This control allows you to verify that the reaction is working properly, that the reaction is not being inhibited by something in the reaction mix, and that the fluorescence signal is being generated and detected. Although PCR-generated and purified amplicons can be use as exogenous positive controls, using a synthetic ultra-long oligo (Synthetic Positive Control –SPCT) is more robust because it provides you with a known copy number.
- b) endogenous positive control (can be run in a separate tube or in multiplex with the experimental sample): For this control, a second target, native to the species of interest and known to be in the experimental sample, is used to normalize the various fluorescent signals obtained from those samples. Most commonly, reference genes are selected for this. Such genes are ubiquitously expressed in all tissues. For example, a list of such reference genes for mouse is found here, and for human, here.



### **Fluorescent Dyes Applications**

Fluorescent dyes are particularly advantageous in biological research because they combine very high sensitivity and selectivity in target detection with low toxicity. As such, they have now become the detection method of choice for tracing the presence of specific biomolecules in cells, cell culture whole organisms, and in vitro assays.

Single-dye labeled oligonucleotides are routinely used as cytogenetic probes in Fluorescence In Situ Hybridization (FISH) to detect and localize specific DNA sequences on chromosomes. FISH is also used to detect and localize specific mRNAs within tissues, thereby revealing spatial-temporal patterns of gene expression within both cells and tissues (1,2). Besides FISH, other common applications of single-dye oligos are as fluorescence-based sequencing and genotyping primers (3), and as probes for oligonucleotide ligation assay (OLA) systems (4), or for SNP detection on microarrays (5).

Dual-dye labeled oligos are particularly useful in fluorescence resonance energy transfer (FRET) experiments for determination of intra- and intermolecular distances at very high resolution (1-10 nm) (6). In addition, dual-labeled oligos containing fluorescent dye/dark quencher pairs are now routinely used in quantitative and qualitative real-time PCR experiments and assays (5'-nuclease assay, Molecular Beacon, Scorpions, etc.) Details of how such dual-labeled probes work for detection of minute amounts of target are found in the Quenchers modifications category.

Fluorescent modifications can also be combined with non-fluorescent modifications in a wide variety of combinations for use in highly specializing applications or research projects.

Click here for a list of fluorophores pricing.

Dye & Quencher Selection Table

Dye & Quencher Selection Table

Click here for a list of fluorophores pricing.



### References

- (1) Carter, N.P. Fluorescence in site hybridization state of the art. Bioimaging (1996), 4: 41-51.
- (2) Maierhofer, C., Jentsch, I., Lederer, G., Fauth, C., Speicher, M.R. Multicolor FISH in two and three dimensions for clastogenic analyses. Mutagenesis (2002), 17: 523-527.
- (3) Giusti, W.G., Adriano, T. Synthesis and characterization of 5'-fluorescent-dye-labeled oligonucleotides. Genome Res. (1993), 2: 223-227.
- (4) Fluorescence-based oligonucleotide ligation assay for analysis of cystic fibrosis transmembrane conductance regulator gene mutations. Hum. Mutat. (1995), 5: 153-165.
- (5) Fan, J.B., Chen, X., Halushka, M.K., Berno, A., Huang, X., Ryder, T., Lipshutz, R.J., Lockhart, D.J., Chakravarti, A. Parallel genotyping of human SNPs using generic high-density oligonucleotide tag arrays. Genome Res. (2000), 10: 853-860.
- (6) Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Design and Applications. Biotechniques (2001), 31: 1106-1121.



### **Modification Code List**

Modification	Code	Catalog Number
2-Amino Purine deoxyribose	[2-AP]	26-6505
Acridine	[Acrd]	26-6694
Atto 390 NHS	[Atto390-N]	26-6951
Atto 425 NHS	[Atto425-N]	26-6952
Atto 430LS NHS	[Atto430LS-N]	26-6953
Atto 465 NHS	[Atto465-N]	26-6954
Atto 488 NHS	[Atto488-N]	26-6955
Atto 490LS NHS	[Atto490LS-N]	26-6956
Atto 495 NHS	[Atto495-N]	26-6957
Atto 514 NHS	[Atto514-N]	26-6958
Atto 520 NHS	[Atto520-N]	26-6959
Atto 532 NHS	[Atto532-N]	26-6960
Atto 540Q NHS	[Atto540Q-N]	26-6962
Atto 550 NHS	[Atto550-N]	26-6963
Atto 565 NHS	[Atto565-N]	26-6964
Atto 575Q NHS	[Atto575Q-N]	26-6969
Atto 590 NHS	[Atto590-N]	26-6971
Atto 594 NHS	[Atto594-N]	26-6973
Atto 610 NHS	[Atto610-N]	26-6974
Atto 612Q NHS	[Atto612Q-N]	26-6975



Atto 620 NHS	[Atto620-N]	26-6976
Atto 633 NHS	[Atto633-N]	26-6978
Atto 643 NHS	[Atto643-N]	26-6612
Atto 647N NHS	[Atto647N-N]	26-6980
Atto 647 NHS	[Atto647N-N]	26-6979
Atto 655 NHS	[Atto655-N]	26-6981
Atto 665 NHS	[Atto665-N]	26-6983
Atto 680 NHS	[Atto680-N]	26-6984
Atto 700 NHS	[Atto700-N]	26-6985
Atto 725 NHS	[Atto725-N]	26-6986
Atto 740 NHS	[Atto740-N]	26-6987
Atto Oxa12 NHS	[AttoOxa12-N]	26-6982
Atto Rho101 NHS	[AttoRho101-N]	26-6970
Atto Rho11 NHS	[AttoRho11-N]	26-6966
Atto Rho12 NHS	[AttoRho12-N]	26-6967
Atto Rho13 NHS	[AttoRho13-N]	26-6972
Atto Rho14 NHS	[AttoRho14-N]	26-6977
Atto Rho3B NHS	[AttoRho3B-N]	26-6965
Atto Rho6G NHS	[AttoRho6G-N]	26-6961
Atto Thio12 NHS	[AttoThio12-N]	26-6968
AZDye-350 NHS	[AZDye-350-N]	26-6471



AZDye-405 NHS	[AZDye-405-N]	26-6485
AZDye-430 NHS	[AZDye-430-N]	26-6486
AZDye-488 NHS	[AZDye-488-N]	26-6571
AZDye-532 NHS	[AZDye-532-N]	26-6481
AZDye-546 NHS	[AZDye-546-N]	26-6488
AZDye-555 NHS	[AZDye-555-N]	26-6529
AZDye-568 NHS	[AZDye-568-N]	26-6708
AZDye-594 NHS	[AZDye-594-N]	26-6478
AZDye-647 NHS	[AZDye-647-N]	26-6479
AZDye-680 NHS	[AZDye-680-N]	26-6716
AZDye-700 NHS	[AZDye-700-N]	26-6596
Cal Fluor Gold 540	[CAL540]	26-6702
CAL Fluor Orange 560	[CAL560]	26-6706
CAL Fluor Red 590	[CAL590]	26-6710
CAL Fluor Red 610	[CAL610]	26-6711
CAL Fluor Red 635	[CAL635]	26-6568
Coumarin Azide	[Cou-N3]	26-6726
Cy2 (Cyanine 2)-NHS	[Cy2-N]	26-7040
Cy3 Internal	[Cy3-Int]	26-6773
Cy3 NHS	[Cy3-N]	26-6998
Cy3-3' (Cyanine 3, 3')	[Cy3-3]	26-6569



Cy3-5' (Cyanine 3)	[Cy3-5]	26-6437
Cy3.5 (Cyanine 3.5) Internal	[Cy3.5-Int]	26-64611
Cy3.5 (Cyanine 3.5-5')	[Cy3.5-5]	26-6461
Cy3B NHS	[Cy3B-N]	26-6907
Cy5 disulfo NHS	[Cy5-S2-N]	26-6443
Cy5 Internal	[Cy5-Int]	26-6774
Cy5 NHS	[Cy5-N]	26-6997
Cy5 (Cyanine 5-3')	[Cy5-3]	26-6549
Cy5 (5'-Cyanine 5)	[Cy5-5]	26-6436
Cy5.5 (Cyanine 5.5)	[Cy5.5-5]	26-6460
Cy5.5 (Cyanine 5.5) Internal	[Cy5.5-Int]	26-64601
Cy7 (Cyanine 7)	[Cy7-N]	26-6474
Cy7.5 (Cyanine 7.5)	[Cy7.5-N]	26-6498
Dansyl-X NHS	[Dnsl-X]	26-6458
DNP TEG (2, 4-dinitrophenyl)	[DNP-TEG]	26-6512
etheno dexoyadenosine dA	[Eth-dA]	26-6506
Fam (6-fluorescein amidite (6-FAM))-3'	[Fam-3]	26-6516
Fam (6-fluorescein amidite (6-FAM))-5'	[Fam-5]	26-6431
Fam (6-fluorescein amidite (6-FAM))-Internal	[Fam-Int]	26-64311
Fluorescein dT (Fam dT)	[Fam-dT]	26-6422
Fam Serinol Internal	[Fam-Ser]	26-6503



Fam-NHS (6-Fam NHS)	[Fam-N]	26-6730
Fam-TEG Azide	[Fam-TEG-N3]	26-6722
Ferrocene-dT	[Fc-dT]	26-6906
Fluorescein-3' (FITC)	[FI-3]	26-6595
Fluorescein-5' (FITC)	[FI-5]	26-6426
Hex-5' (Hexachloro-Fluorescein)	[Hex-5]	26-6432
Hex-Azide-6	[Hex-N3]	26-6723
HEX-NHS (Hexachloro-Fluorescein)	[Hex-N]	26-6590
HEX-SIMA dT	[HEX-SIMA-dT]	26-6499
Hyper5	[Hyper5]	26-6705
IRDye 650LT-NHS	[IRD650LT-N]	26-6646
IRDye 680-NHS	[IRD680RD-N]	26-6647
IRDye 700	[IRD700]	26-6672
IRDye 700-NHS	[IRD700-N]	26-6766
IRDye 750-NHS	[IRD750-N]	26-6648
IRDye 800	[IRD800]	26-6673
IRDye 800-NHS	[IRD800RS-N]	26-6767
Joe (4-5-Dichloro carboxy fluorescein)	[JOE]	26-6467
LC Cyan 500 NHS	[LC-C500-N]	26-6688
LC Red 610 NHS	[LC610-N]	26-6675
LC Red 640 NHS	[LC640-N]	26-6677



MarBlue-460NHS	[MarBI-460-N]	26-6687
Methylene Blue (MB2-Azide)	[MB-N3]	26-6988
Methylene Blue II	[MB-II]	26-6909
Methylene Blue Mal (MB2-Mal)	[MB2-Mal]	26-6526
Methylene blue MB2-NHS	[MB2-N]	26-6908
PBlue-455 NHS	[PBlue-455]	26-6524
Quasar 570-3'	[Qsr-570]	26-6567
Quasar 670-3'	[Qsr-670]	26-6709
ROX (6-Carboxyl-X-Rhodamine) NHS	[ROX-N]	26-6430
Tamra NHS	[Tamra-N]	26-6450
TAMRA-3' (Carboxytetramethylrhodamine)	[3-Tamra]	26-6451
TAMRA-5' (Carboxytetramethylrhodamine)	[5-Tamra]	26-6947
Tamra-dT (Carboxytetramethylrhodamine-dT)	[Tamra-dT]	26-6449
Tet-5' (Tetrachloro-Fluorescein)	[Tet-5]	26-6433
Tet Azide	[Tet-N3]	26-6724
Tet-NHS (Tetrachloro-Fluorescein)	[Tet-N]	26-6594
Tide Fluor 5 NHS	[TF5-N]	26-6604
TXRed-616 NHS	[TXRed-616-N]	26-6469
Yakima Yellow Epoch	[YYel-3]	26-6700T
Yakima Yellow Epoch	[YYel-5]	26-6700





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### 2-Amino Purine

Category	Others		
Modification Code	2-AP		N-
Reference Catalog Number	26-6505	5'- Oligo	
5 Prime	Υ	o= -o-	NH <sub>2</sub>
3 Prime	Υ	ÓH	
Internal	Υ	2-Amino Purine	
Molecular Weight(mw)	313.21	[26-6505-XX]	=P-0- www Oligo -3'
			OH

- 2-Amino Purine (2-AP) is a mildly fluorescent molecule that is classified as an adenine and guanine analog, and thus can pair with both thymine and cytosine bases (1,2). It is an attractive choice for use as a probe in nucleic acid secondary structural studies, both because its fluorescence is highly sensitive to the nature of the local environment, and because it usually does not significantly affect duplex stability (3). Examples include the hairpin-loop structure of the (CAG)8 repeat, involved in several neurodegenerative disorders 2-AP substituted for A (4), the G-quadruplex telomeric structure [AGGG(TTAGGG)3] 2-AP substitute for A (5). 2-AP also has been used to characterize the effects of DNA mismatch repair on mutagenesis induced by several different nucleoside analogs (6). **References**
- 1. Jean JM, Hall KB (2001). "2-Aminopurine fluorescence quenching and lifetimes: role of base stacking". Proc. Natl. Acad. Sci. U.S.A. 98 (1): 37-41. doi:10.1073/pnas.011442198.
- 2. Negishi, K.; Bessho, T.; Hayatsu, H. Nucleoside and nucleobase analog mutagens. Mutat. Res. (1994), 318: 227-238.
- 3. Ballin, J.D., et al. Local RNA Conformational Dynamics Revealed by 2-Aminopurine Solvent Accessibility. *Biochemistry* (2008), **47**: 7043-7052.
- 4. Degtyareva, N.N.; Reddish, M.J.; Sengupta, B.; Petty, J.T. Structural Studies of a Trinucleotide Repeat Sequence Using 2-Aminopurine. *Biochemistry* (2009), **48**: 2340-2346.
- 5. Kimura, T.; Kawai, K.; Fujitsuka, M.; Tetsuro, M. Monitoring G-quadruplex structures and G-quadruplex-ligand complex using 2-aminopurine modified oligonucleotides. *Tetrahedron* (2007), **63**: 3585-3590.
- 6. Negishi, K.; et al. Binding specificities of the mismatch binding protein, MutS, to oligonucleotides containing modified bases. *Nucleic Acids Res. Supplement No. 1* (2001), 221-222.



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# Oligo Modifications

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### **Acridine**

Category	Intercalators		
Modification Code	Acrd	N CI	
Reference Catalog Number	26-6694		
5 Prime	Υ	MeO	
3 Prime	Υ	HN	$\wedge$
Internal	Υ	· · · · · · · · · · · · · · · · · · ·	`ОН
Molecular Weight(mw)	450.86	5' Acridine [26-6694-XX]	
		20000000	(P)—wwwOligo-3'

Acridine is classified as a DNA intercalating agent. Labeling of DNA oligos with acridine allows them to rapidly and stably intercalate into a target dsDNA molecule, adding increased stability to the double helix (1). In addition, incorporation of acridine to the 3'-end of an oligo confers a high level of exonuclease resistance to that end (2). Thus, oligos labeled with acridine may be useful in applications involving oligo hybrids requiring higher levels of stability.

Acridine-labeled oligos containing a polypyrimidine sequence possess the ability to form triplex helices that are highly stable, and, due to their increased hydrophobicity, can pass through membranes more easily than normal oligos. Such oligos were used as anti-sense reagents to suppress c-myc protooncogene expression and control tumor growth in mice (3), and as a transcriptional repressor to the IL-2 Receptor (4).

Since acridine is a fluorescent dye, it can also be use to make dye-labeled oligos. An interesting such application is the use of acridine-labeled primers to study non-enzymatic-template-directed RNA synthesis to provide experimental support for theories concerning possible replication of genetic information by early life forms on Earth (5). **References** 

- 1. Fukui, K., Tanaka, K. The Acridine Ring Selectively Intercalated into a DNA Helix at Various Types of Abasic Sites: Double Strand Formation and Photophysical Properties. *Nucleic Acids Res.* (1996), **24**: 3962-3967.
- 2. Gamper, H.B., Reed, M.W., Cox, T., Virosco, J.S., Adams, A.D., Gall, A.A., Scholler, J.K., Meyer, R.B. Facile preparation of nuclease resistant 3� modified oligodeoxynucleotides. *Nucleic Acids Res.* (1993), **21**: 145-150.
- 3. Stewart, D.A., Xu, Xiaohou, Thomas, S.D., Miller, D.M. Acridine-modified, clamp-forming antisense oligonucleotides synergize with cisplatin to inhibit c-Myc expression and B16-F0 tumor progression. *Nucleic Acids Res.* (2002), **30**: 2565-2574.
- 4. Grigoriev, M., Praseuth, D., Robin, P., Hemar, A., Saison-Behmoaras, T., Dautry-Varsat, A., Thuong, N.T., Helene, C., Harel-Bellan, A. A Triple-Helix-forming Oligonucleotide-Intercalator Conjugate Acts as Transcriptional Repressor via Inhibition of NFkB Binding to Interleukin-2 Receptor alpha-Regulatory Sequence. *J. Biol. Chem.* (1992), **267**: 3389-3395.
- 5. Kurz, M., Gobel, K., Hartel, C., Gobel, M.W. Acridine-labeled primers as tools for the study of nonenzymatic RNA oligomerization.



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Helv. Chim. Acta (1998), 81: 1156-1180.





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### **Atto 390-N**

Category	Fluorescent Dyes	
Modification Code	Atto390-N	
Reference Catalog Number	26-6951	H <sub>3</sub> C
5 Prime	Υ	H <sub>3</sub> C
3 Prime	Υ	
Internal	Υ	NH-www Oligo 3' or 5'
Molecular Weight(mw)	440	Atto 390 [26-6951]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.





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### **Atto 425-N**

Category	Fluorescent Dyes	O 
Modification Code	Atto425-N	
Reference Catalog Number	26-6952	H <sub>3</sub> C
5 Prime	Υ	H <sub>3</sub> C N O O
3 Prime	Υ	
Internal	Υ	NH-www Oligo 3' or 5'
Molecular Weight(mw)	498	Atto 425 [26-6952]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.





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### Atto 430LS-N

Category Fluorescent Dyes

Modification Code Atto430LS-N

Reference Catalog Number 26-6953

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 686

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Atto 465-N

Category	Fluorescent Dyes	
Modification Code	Atto465-N	
Reference Catalog Number	26-6954	H <sub>2</sub> N NH <sub>2</sub>
5 Prime	Υ	
3 Prime	Υ	
Internal	Υ	NHvvvv·Oligo 3' or 5'
Molecular Weight(mw)	493	Atto 465 [26-6954]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.





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### Oligo Modifications

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#### Atto 488-N

Category	Fluorescent Dyes	
Modification Code	Atto488-N	Olive 21 a
Reference Catalog Number	26-6955	NH—www Oligo 3' o
5 Prime	Υ	
3 Prime	Υ	Atto 488
Internal	Υ	[26-6955]
Molecular Weight(mw)	981	H-N SO <sub>3</sub> H SO <sub>3</sub> H H

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.





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## Oligo Modifications

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#### Atto 490LS-N

Category Fluorescent Dyes

Modification Code Atto490LS-N

Reference Catalog Number 26-6956

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 793

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



ATTO 490LS is a new fluorescent label featuring an extraordinary large Stokes-shift of 165 nm. Thus the emission spectrum is almost completely separated from the absorption spectrum, making the dye highly suitable for multicolor experiments, in particular in combination with ATTO 488 and ATTO 514. ATTO 490LS is very hydrophilic, highly water soluble, and exhibits a relatively high fluorescence quantum yield, which is only slightly reduced on conjugation to biomolecules, e.g. proteins, even at high degrees of labeling (DOL).

ATTO 490LS is an anionic dye. After conjugation to a substrate the dye carries a net electrical charge of -1. The fluorescence is excited most efficiently in the range 460 - 530 nm.

Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.





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## Oligo Modifications

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#### Atto 495-N

Category	Fluorescent Dyes	
Modification Code	Atto495-N	
Reference Catalog Number	26-6957	N+
5 Prime	Υ	N N
3 Prime	Υ	
Internal	Υ	NH—www.Oligo 3' or 5
Molecular Weight(mw)	1111	Atto 495 [26-6957]

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



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### **Atto 5140N**

#### Click here for a list of fluorophores.

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### **Atto 520-N**

#### Click here for a list of fluorophores.

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### Oligo Modifications

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### **Atto 532-N**

Category	Fluorescent Dyes	
Modification Code	Atto532-N	
Reference Catalog Number	26-6960	NH—www Oligo 3' or !
5 Prime	Υ	
3 Prime	Υ	Atto 532
Internal	Υ	N>H [26-6960]
Molecular Weight(mw)	1081	SO <sub>3</sub> H SO <sub>3</sub> H

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



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### Oligo Modifications

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### Atto 540Q-N

Category Fluorescent Dyes

Modification Code Atto540Q-N

Reference Catalog Number 26-6962

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 756

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



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### Oligo Modifications

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### **Atto 550-N**

Category Fluorescent Dyes

Modification Code Atto550-N

Reference Catalog Number 26-6963

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 791

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







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## Oligo Modifications

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### **Atto 565-N**

Category Fluorescent Dyes

Modification Code Atto565-N

Reference Catalog Number 26-6964

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 708

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







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## Oligo Modifications

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### Atto 575Q-N

Category Fluorescent Dyes

Modification Code Atto575Q-N

Reference Catalog Number 26-6969

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 787

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



ATTO 575Q is a novel fluorescence quencher (energy acceptor in FRET process). Characteristic features of the label are strong absorption and high thermal and photo-stability. The dye is moderately hydrophilic. ATTO 575Q is a cationic dye. Contrary to ATTO 580Q, ATTO 575Q is supplied as single isomer. After coupling to a substrate the dye carries a net electrical charge of +1.

ATTO 575Q has an absorption maximum at 582 nm (H2O). Atto 580Q is characterized by a high photostability and thermostability. Ato 575Q can be utilized as a fluorescence quencher ( $\lambda$  absorbance = 582 nm) on amine-labeled nucleotides for FRET experiments. Works well in combination with the following fluorescent dyes: TAMRA, ATTO 550, ATTO 565, ATTO 590, and ROX.





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## Oligo Modifications

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### **Atto 590-N**

Category Fluorescent Dyes

Modification Code Atto590-N

Reference Catalog Number 26-6971

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 788

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







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## Oligo Modifications

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### Atto 594-N

Category Fluorescent Dyes

Modification Code Atto594-N

Reference Catalog Number 26-6973

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 1389

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







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## Oligo Modifications

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### **Atto 610-N**

Category	Fluorescent Dyes	
Modification Code	Atto610-N	-N
Reference Catalog Number	26-6974	
5 Prime	Υ	Н
3 Prime	Υ	ATTO 610
Internal	Υ	(Atto610)
Molecular Weight(mw)	588	[26-6974]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







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## Oligo Modifications

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### Atto 612Q-N

Category Fluorescent Dyes

Modification Code Atto612Q-N

Reference Catalog Number 26-6975

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 888

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Atto 620-N**

Category Fluorescent Dyes

Modification Code Atto620-N

Reference Catalog Number 26-6976

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 709

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Atto 633-N**

Category Fluorescent Dyes

Modification Code Atto633-N

Reference Catalog Number 26-6978

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 749

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Atto 643-N**

Category Fluorescent Dyes

Modification Code Atto643-N

Reference Catalog Number 26-6612

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 534.7

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Atto 647-N**

Category	Fluorescent Dyes	
Modification Code	Atto647N-N	
Reference Catalog Number	26-6980	
5 Prime	Υ	
3 Prime	Υ	$\gamma_{-}$
Internal	Υ	5' or 3' Oligo www-NH Atto 647N
Molecular Weight(mw)	628	[26-6980]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO 647N belongs to a new generation of fluorescent labels for the red spectral region. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, and **exceptionally high stability towards atmospheric ozone**. Thus ATTO 647N is highly suitable for single-molecule detection applications and high-resolution microscopy such as SIM, STED etc. Additionally the dye highly qualifies to be applied in flow cytometry (FACS), fluorescence in-situ hybridization (FISH) and many more.

In common with most ATTO-labels, absorption and fluorescence are independent of pH, at least in the range of pH 2 to 11, used in typical applications. The dye is moderately hydrophilic. ATTO 647N is a cationic dye. After coupling to a substrate the dye carries a net electrical charge of +1. As supplied ATTO 647N consists of a mixture of two isomers with practically identical absorption and fluorescence properties. The fluorescence is excited most efficiently in the range 625 - 660 nm. A suitable excitation source is the 647 nm line of the Krypton-lon laser or a diode-laser emitting at 650 nm. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

**Extinction Coefficient\*** 



Color**	
Quencher	
Cy5 650 665 250,000	
IRDye 650 NHS 650 665 230,000	
AZ647 NHS 655 680 191,800	
AZ680 NHS 678 701 185,000	
Cy5.5 684 710 198,000	
IRDye 700 NHS 684 710 288,000	
AZdye700 NHS 696 719 192,000	
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Atto 700 NHS 700 716 120,000
Atto 725 NHS 728 751 120,000
Atto 740 NHS 743 763 120,000
Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
$^{*}$ Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.
Click here for a list of fluorophores.



Click here for list of quenchers.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Atto 647N-N

Category Fluorescent Dyes

Modification Code Atto647N-N

Reference Catalog Number 26-6979

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 811

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Atto 655-N**

Category Fluorescent Dyes

Modification Code Atto655-N

Reference Catalog Number 26-6981

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 887

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Atto 665-N**

Category Fluorescent Dyes

Modification Code Atto665-N

Reference Catalog Number 26-6983

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 820

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Atto 680-N**

Category Fluorescent Dyes

Modification Code Atto680-N

Reference Catalog Number 26-6984

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 828

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **Atto 700-N**

Category Fluorescent Dyes

Modification Code Atto700-N

Reference Catalog Number 26-6985

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 547.7

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

ATTO 700 belongs together with ATTO 655 and ATTO 680 to a new generation of fluorescent labels. Characteristic features of the label are strong absorption, high fluorescence quantum yield, good water solubility, and high thermal and photo-stability. ATTO 700 is a zwitterionic dye. After coupling to a substrate the dye moiety is electrically neutral. ATTO 700 is a strong electron acceptor. Its fluorescence is efficiently quenched by electron donors like guanine, tryptophan, etc. The fluorescence is excited most efficiently in the range 670 - 715 nm.

Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation.



The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. NHS Ligand conjugation requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Maleimide Ligand conjugation requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

**Extinction Coefficient\*** 

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000



AZ647 NHS 655 680 191,800	
AZ680 NHS 678 701 185,000	
Cy5.5 684 710 198,000	
IRDye 700 NHS 684 710 288,000	
AZdye700 NHS 696 719 192,000	
Atto 700 NHS 700 716 120,000	
Atto 725 NHS 728 751 120,000	
Atto 740 NHS 743 763 120,000	



Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
$^*$ Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.
Click here for a list of fluorophores.
Click here for list of quenchers.



Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### **Atto 725-N**

Category Fluorescent Dyes

Modification Code Atto725-N

Reference Catalog Number 26-6986

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 613

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

ATTO 725 is a pH sensitive product. While practically stable up to pH 7.4, it slowly degrades at higher pH.

ATTO 725 together with ATTO 740 belongs to a new generation of fluorescent labels for the near infrared spectral region. Characteristic features of the dye are strong absorption and good fluorescence as well as excellent thermal and photo-stability. ATTO 725 is a cationic dye. After coupling to a substrate the dye carries a net electrical charge of +1. The fluorescence is excited most efficiently in the range 700 - 745 nm.

Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer.



Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. NHS Ligand conjugation requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click a wide selection of thiol modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click

here for a list of conjugation chemistry modifications. Maleimide Ligand conjugation requires a thiol group. Gene Link offers here for a list of click chemistry modifications.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide Fluorophore Name Absorbance Max, nm +/-10 Emission Max, nm +/-10 Extinction Coefficient\* Color\*\* Quencher Cy5 650 665 250,000 IRDye 650 NHS 650 665 230,000 AZ647 NHS 655 680 191.800

AZ680 NHS 678 701 185,000



Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000 IRDye 750 NHS 756 776 260,000



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IRDye 800 NHS 795 819 240,000

 $^*$  Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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#### **Atto 740-N**

Category Fluorescent Dyes

Modification Code Atto740-N

Reference Catalog Number 26-6987

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 450.6

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

ATTO 740 is a pH sensitive product. While practically stable up to pH 7.4, it slowly degrades at higher pH.

ATTO 740 together with ATTO 725 belongs to a new generation of fluorescent labels for the near infrared spectral region. Characteristic features of the dye are strong absorption and good fluorescence as well as excellent thermal and photo-stability. ATTO 740 is a cationic dye. After coupling to a substrate the dye carries a net electrical charge of +1. The fluorescence is excited most efficiently in the range 720 - 755 nm.

Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation.



The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. NHS Ligand conjugation requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Maleimide Ligand conjugation requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

**Extinction Coefficient\*** 

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000



AZ647 NHS 655 680 191,800	
AZ680 NHS 678 701 185,000	
Cy5.5 684 710 198,000	
IRDye 700 NHS 684 710 288,000	
AZdye700 NHS 696 719 192,000	
Atto 700 NHS 700 716 120,000	
Atto 725 NHS 728 751 120,000	
Atto 740 NHS 743 763 120,000	



Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
$^*$ Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.
Click here for a list of fluorophores.
Click here for list of quenchers.



Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Atto Oxa12-N

Category Fluorescent Dyes

Modification Code AttoOxa12-N

Reference Catalog Number 26-6982

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 835

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Atto Rho101-N

Category Fluorescent Dyes

Modification Code AttoRho101-N

Reference Catalog Number 26-6970

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 892

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



ATTO Rho101 is a derivative of the well-known dye Rhodamine 101. Characteristic features of the label are strong absorption, extraordinarily high fluorescence quantum yield, and high thermal and photo-stability. The dye is moderately hydrophilic. ATTO Rho101 is a cationic dye. After coupling to a substrate the dye carries a net electrical charge of +1.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Atto Rho11-N

Category Fluorescent Dyes

Modification Code AttoRho11-N

Reference Catalog Number 26-6966

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 763

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Atto Rho12-N

Category Fluorescent Dyes

Modification Code AttoRho12-N

Reference Catalog Number 26-6967

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 847

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Atto Rho13-N

Category Fluorescent Dyes

Modification Code AttoRho13-N

Reference Catalog Number 26-6972

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 843

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Atto Rho14-N

Category Fluorescent Dyes

Modification Code AttoRho14-N

Reference Catalog Number 26-6977

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 981

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Atto Rho3B-N

Category Fluorescent Dyes

Modification Code AttoRho3B-N

Reference Catalog Number 26-6965

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 739

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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#### Atto Rho6G-N

Category Fluorescent Dyes

Modification Code AttoRho6G-N

Reference Catalog Number 26-6961

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 711

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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### Atto Thio12-N

Category	Fluorescent Dyes	
Modification Code	AttoThio12-N	
Reference Catalog Number	26-6968	NH—www Oligo 3' or
5 Prime	Υ	O
3 Prime	Υ	
Internal	Υ	Atto Thio12 [26-6968]
Molecular Weight(mw)	699	_N <sup>+</sup>

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



ATTO Thio12 is a new label closely related to the well-known rhodamines. The characteristic feature of the label is high yield of triplet formation. The dye is moderately hydrophilic. Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection.

ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### AZDye-350-N

Category Fluorescent Dyes

Modification Code AZDye-350-N

Reference Catalog Number 26-6471

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 410.35

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

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#### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), **47**: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

#### References

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- 2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995). **4**: 1-6.
- 3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





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### Oligo Modifications

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### AZDye-405-N

Category Fluorescent Dyes

Modification Code AZDye-405-N

Reference Catalog Number 26-6485

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 510

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



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### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), **47**: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

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### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### AZDye-430-N

Category Fluorescent Dyes

Modification Code AZDye-430-N

Reference Catalog Number 26-6486

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 585

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

#### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), **47**: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

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### Oligo Modifications

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### AZDye-488-N

Category Fluorescent Dyes

Modification Code AZDye-488-N

Reference Catalog Number 26-6571

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 833.26

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



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Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

## References

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# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## AZDye-532-N

Category Fluorescent Dyes

Modification Code AZDye-532-N

Reference Catalog Number 26-6481

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 723.8

## Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



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#### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), **47**: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

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# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## AZDye-546-N

Category Fluorescent Dyes

Modification Code AZDye-546-N

Reference Catalog Number 26-6488

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 557

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

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#### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), **47**: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

### References

1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.



- 2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## AZDye-555-N

Category Fluorescent Dyes

Modification Code AZDye-555-N

Reference Catalog Number 26-6529

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 1247.64

## Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), **47**: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

### References

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- 2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.*



(1995), **4**: 1-6.

3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.

4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





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# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## AZDye-568-N

Category Fluorescent Dyes

Modification Code AZDye-568-N

Reference Catalog Number 26-6708

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 791

## Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

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#### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), **47**: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

### References

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- 2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995). **4**: 1-6.
- 3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





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# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## AZDye-594-N

Category Fluorescent Dyes

Modification Code AZDye-594-N

Reference Catalog Number 26-6478

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 819.85

## Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

Royalty charges are additional for Alexa dyes.

### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), 47: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

### References

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# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## AZDye-647-N

Category Fluorescent Dyes

Modification Code AZDye-647-N

Reference Catalog Number 26-6479

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 970.1

## Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



AZDye 647 Abs Maxima is at 655 nm and Emission Maxima is at 680 nm.

Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes. AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

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Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

**Extinction Coefficient\*** 

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000



AZ647 NHS 655 680 191,800	
AZ680 NHS 678 701 185,000	
Cy5.5 684 710 198,000	
IRDye 700 NHS 684 710 288,000	
AZdye700 NHS 696 719 192,000	
Atto 700 NHS 700 716 120,000	
Atto 725 NHS 728 751 120,000	
Atto 740 NHS 743 763 120,000	



Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
$^*$ Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.
Click here for a list of fluorophores.
Click here for list of quenchers.
References 1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. <i>J. Histochem. Cytochem.</i> (1999), 47: 1179-1188.
Click here for list of quenchers.
Click here for a list of fluorophores.
Quencher Spectral Data
Quencher
Absorption Max, nm



Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

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- 3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
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Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## AZDye-680-N

Category Fluorescent Dyes

Modification Code AZDye-680-N

Reference Catalog Number 26-6716

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 924.21

## Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes. AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

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Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

**Extinction Coefficient\*** 

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800



AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000



Cy7 NHS 740 773 199,000	
IRDye 750 NHS 756 776 260,000	
cy7.5 NHS 788 808 223,000	

IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

References

- 1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), 31: 1106-1121.
- 2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), 4: 1-6.
- 3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), 28: 3752-3761.
- 4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## AZDye-700-N

Category Fluorescent Dyes

Modification Code AZDye-700-N

Reference Catalog Number 26-6596

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 924.21

## Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Azdye-700 NHS has the same chemical structure and spectral properties as of Alexa Fluor® 700 (Alexa Fluor® is the trademark of ThermoFisher).

Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes. AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.

-

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient\*

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000



AZ647 NHS 655 680 191,800 AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000



Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
* Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

### References

- 1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), 31: 1106-1121.
- 2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), 4: 1-6.
- 3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), 28: 3752-3761.
- 4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## Cal Fluor Gold 540

Category Fluorescent Dyes

Modification Code CAL540

Reference Catalog Number 26-6702

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 532.52

## Click here for a list of fluorophores.

CAL Fluor® Gold 540 dye fluoresces in the yellow green region of the visible spectrum. CAL Fluor Gold 540 amidite is used for the 5' labeling of fluorogenic probes used in 5' nuclease assays, Molecular Beacons, Scorpions® primers and other detection assays. This amidite does not contain a DMT protecting group and can only be added to the 5' terminus of the oligo. CAL Fluor Gold 540 fluorophore is an alternative for TET and is quenched by BHQ®-1.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## **CAL Fluor Orange 560**

Category Fluorescent Dyes

Modification Code CAL560

Reference Catalog Number 26-6706

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 560.6

### Click here for a list of fluorophores.

-CAL Fluor Orange 560 is an dye which fluoresces in the yellow-orange region of the visible spectrum. CAL Fluor Orange 560 is used for the 5'-labeling of fluorogenic probes used in 5' nuclease assays, Molecular Beacons, and other genomic assays. BHQ-1 will quench the CAL Fluor Orange 560 moiety. CAL Fluor Orange 560 is an alternative for VIC and HEX. Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.5 Red 588 604 ROX Red 575 602 CAL Fluor Orange 590 Red 569 591 Texas Red Red 583 603



genelink.com/oligo\_modifications\_reference/OMR\_mod\_category\_intro.asp?mod\_sp\_cat\_id=18"> Click here for a list of fluorophores.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## **CAL Fluor Red 590**

Category Fluorescent Dyes

Modification Code CAL590

Reference Catalog Number 26-6710

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 588.65



### Click here for a list of fluorophores.

CAL Fluor Red 590 is a fluorescent dye manufactured by Biosearch Technologies, it fluoresces in the yellow-orange region of the visible spectrum. CAL Fluor Red 590 is used for the 5' labeling of fluorogenic probes used in 5' nuclease assays, Molecular Beacons, Scorpion primers, Amplifluor primers, and other genomic assays. CAL Fluor Red 590 is an alternative dye for TAMRA and is quenched by BHQ-2 dye. Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.5 Red 588 604 ROX Red 575 602 CAL Fluor Red 590 Red 569 591 Texas Red Red 583 603





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## **CAL Fluor Red 610**

Category Fluorescent Dyes

Modification Code CAL610

Reference Catalog Number 26-6711

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 636.7

## Click here for a list of fluorophores.

CAL Fluor Red 610 is a fluorescent dye manufactured by Biosearch Technologies. CAL Fluor Red 610 fluoresces in the orange-red region of the visible spectrum. CAL Fluor Red absorption max is at 590 nm and emission max is at 610 nm with Extinction Coefficient at Lambda max: 108000/M/cm.

It is used for the 5'- labeling of fluorogenic probes used in 5' nuclease assays, Molecular Beacons, and other detection assays. This fluorescent dye can only be added to the 5' terminus. BHQ-2 dye will quench the CAL Fluor Red 610 moiety. The CAL Fluor Red 610 fluorophore is coupled directly to an oligo and is an alternative for Texas Red dye with absorption max is at 589 nm and emission max at 615 nm.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **CAL Fluor Red 635**

Category Fluorescent Dyes

Modification Code CAL635

Reference Catalog Number 26-6568

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 760.7



### Click here for a list of fluorophores.

CAL Fluor® Red 635 is a fluorescent dye manufactured by Biosearch Technologies, it fluoresces in the orange-red region of the visible spectrum. CAL Fluor Red 635 is used for the 5' labeling of fluorogenic probes used in 5' nuclease assays, Molecular Beacons, Scorpions® Primers, Amplifluor® Primers, and other genomic assays. It only be added to the 5' terminus of the oligo. BHQ®-2 dye will quench the CAL Fluor Red 635 moiety. CAL Fluor Red 635 is an alternative for LC Red 640® dye.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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### **Coumarin Azide**

Category Click Chemistry

Modification Code Cou-N3

Reference Catalog Number 26-6726

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 203.15

HO O O O

Coumarin Azide [26-6726-XX]

This modification is a post synthesis conjugation to an alkyne or DBCO modification at the appropriate site for click conjugation.

Coumarin (7-Hydroxycoumarin)-Azide is a fluorescent dye containing a terminal azide group. Coumarin is also known as umbelliferone. Coumarin is highly fluorescent and pH-sensitive, with an absorbance maximum of 358 nm and an emission maximum of 480 nm; thus it emits in the blue region of the visible spectrum. The presence of the azide allows the user to use "Click Chemistry" (a [3+2] cycloaddition reaction between alkynes and azides, using copper (I) iodide as a catalyst) to conjugate the Coumarin-Azide to a terminal alkyne-modified oligo with extremely high regioselectivity and efficiency (1,2). Preparation of the alkyne-modified oligo can be achieved using the 5'-Hexynyl modifier (see its respective tech sheet for details). Because coumarin is effectively quenched if its hydroxyl group is either alkylated or phosphorylated, it is useful in high-throughput screening for enzyme lipases and phosphatases. **References** 

- 1. Huisgen, R. Angew. Chem. Int. Ed. (1963), 2: 565-568.
- 2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.

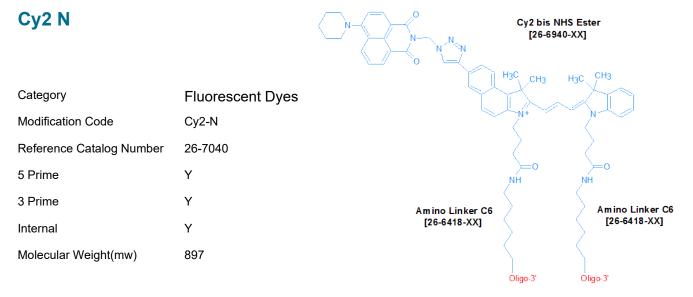




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### Oligo Modifications

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#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Cy2 is a bright fluorescent dye that emits in the visible spectrum and can be used to label oligonucleotides. Cy2 has an absorbance maximum of 492 nm and an emission maximum of 510 nm. Cy2 is most commonly used to label the internal standard when such is required in an experiment (for example, difference gel electrophoresis (DIGE).





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## Oligo Modifications

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### Cy3 Internal

Category Fluorescent Dyes H<sub>3</sub>C CH<sub>3</sub> H<sub>3</sub>C Modification Code Cy3-Int Reference Catalog Number 26-6773 5 Prime 3 Prime Υ Internal Υ Molecular Weight(mw) 618 Cy3 Internal Label [26-6773-XX] [Cy3-Int]

### Click here for a list of fluorophores.

Cyanine 3 (Cy3) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3 is reactive, water-soluble, and has an absorbance maximum of 550 nm and an emission maximum of 570 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy3 plays a particularly important role in real-time PCR applications, being used as the reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy3 is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

Cy3 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy3 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH 0 strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy3-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility.

Applied Biosystems Proprietary Dyes & Possible Substitutions

Fluorophore

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.



### Click here for a list of fluorophores.

#### References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.
- 4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), **76**: 922-926.





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## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Cy3 NHS

Category	Fluorescent Dyes	
Modification Code	Cy3-N	
Reference Catalog Number	26-6998	CI CI
5 Prime	Υ	N
3 Prime	Υ	Cy3 NHS [26-6998-XX]
Internal	Υ	
Molecular Weight(mw)	474.2	Amino linker  Oligo-3'NH

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Cyanine 3 (Cy3) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3 is reactive, water-soluble, and has an absorbance maximum of 550 nm and an emission maximum of 570 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy3 plays a particularly important role in real-time PCR applications, being used as the reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy3 is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

Cy3 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy3 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy3-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility.

Reaction scheme for primary amine labelled oligos with NHS ester is shown in the figure below.

#### - References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
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# Oligo Modifications

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### Cy3-3'

		H3C CH3	H <sub>3</sub> C CH3
Category	Fluorescent Dyes		
Modification Code	Cy3-3		
Reference Catalog Number	26-6569	V 1N+	N. «
5 Prime	N	(	$\rangle$
3 Prime	Υ	$\rangle$	
Internal	N	НО	<u>O</u>
Molecular Weight(mw)	606.71	5'-Cy3 Label	0=P-0Oligo-3'
		[26-6437-XX]	ОН

### Click here for a list of fluorophores.

Prices listed above are for 5' modification. Internal and 3' incurs additional charges and are post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Cyanine 3 (Cy3) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3 is reactive, water-soluble, and has an absorbance maximum of 550 nm and an emission maximum of 570 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy3 plays a particularly important role in real-time PCR applications, being used as the reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy3 is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

Cy3 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy3 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH 0 strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy3-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility. **References** 

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A.



- , Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308. 4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), **76**: 922-926.





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### Oligo Modifications

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### Cy3-5'

Category Fluorescent Dyes

Modification Code Cy3-5

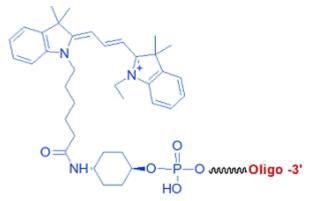
Reference Catalog Number 26-6437

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 630.78



Cy3-5' [26-6437-XX]

#### Click here for a list of fluorophores.

Prices listed above are for 5' modification. Internal and 3' incurs additional charges and are post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Cyanine 3 (Cy3) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3 is reactive, water-soluble, and has an absorbance maximum of 550 nm and an emission maximum of 570 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy3 plays a particularly important role in real-time PCR applications, being used as the reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy3 is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

Cy3 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy3 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH 0 strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy3-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility.

Applied Biosystems Proprietary Dyes & Possible Substitutions

Fluorophore

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.



### Click here for a list of fluorophores.

#### References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
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### Oligo Modifications

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### Cy3.5 Internal

Cyanine 3.5 (Cy3.5) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3.5 is reactive, water-soluble, and has an absorbance maximum of 581 nm and an emission maximum of 596 nm. It is available as a phosphoramidite, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy3.5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy3.5 is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

Cy3 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH).

Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.



5 Red 588 604 ROX Red 575 602 CAL Fluor Red 590 Red 569 591 Texas Red Red 583 603

### Click here for a list of fluorophores.

#### References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
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## Oligo Modifications

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Cy3.5-5'

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Cy3 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH).

Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.



5 Red 588 604 ROX Red 575 602 CAL Fluor Red 590 Red 569 591 Texas Red Red 583 603

### Click here for a list of fluorophores.

#### References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Cy3B-N

Category	Fluorescent Dyes	5'-Amino Linker C [26-6418-XX]
Modification Code	Cy3B-N	
Reference Catalog Number	26-6907	SO <sub>3</sub> H <sub>3</sub> C CH <sub>3</sub> H <sub>3</sub> C CH <sub>3</sub> NH
5 Prime	Υ	
3 Prime	Υ	N+ N
Internal	Υ	0
Molecular Weight(mw)	678	5'-Cy3B NHS Ester [26-6907-XX]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Cyanine 3B (Cy3B) is an orange fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy3B is reactive, water-soluble, has an absorbance maximum of 559 nm and an emission maximum of 570 nm. It is available as an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cyanine dyes normally are capable of cis/trans isomerization around the polymethine, which can lead to loss of fluorescence after excitation, and weaker signal. By contrast, Cy3B is conformationally locked, the dye is not subject to photo-isomerization and improved fluorescent properties (1,2). Consequently, Cy3B is both extremely bright and extremely stable. Cy3B can be used to substitute in any oligonucleotide-based application suitable for Cy3, such as TaqMan probes, Scorpion primers, Molecular Beacons, or Fluorescent In-Situ Hybridization. Like Cy3, Cy3B is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap. **References** 

- 1. Cooper, M., Ebner, A., Briggs, M., Burrows, M., Gardner, N., Richardson, R., West, R. Cy3B: improving the performance of cyanine dyes. *J. Fluoresc.* (2004), **14**: 1145-150.
- 2. Hall, L.M., Gerowska, M., Brown, T. A highly fluorescent DNA toolkit: synthesis and properties of oligonucleotides containing new Cy3, Cy5, and Cy3B monomers. *Nucleic Acids Res.* (2012), **40**: e108.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Cy5 disulfo NHS

Category Fluorescent Dyes Modification Code Cy5-S2-N Na<sup>T</sup>-O<sub>3</sub>S Reference Catalog Number 26-6443 5 Prime 3 Prime Υ Cy5 disulfo NHS Internal Υ [26-6443-XX] Molecular Weight(mw) 533.63 Amino linker

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne: Azide, Azide: DBCO, BCN: Azide, BCN: TCO: Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

The diSulfo Cy5 NHS Ester is a hydrophilic version of Cy5 due to the two sulfo groups. This version is particularly helpful when the standard hydrophobic Cy5 version is not appropriate for the desired application. All NHS ester derivative modifications are post synthesis for oligos and requires a primary amino group on the oligo for conjugation. The amino group can be placed at either the 5' or 3' ends an internally as well.

Cy5 can be used as a replacement for Alexa Fluor 647 Succinimidyl Ester, DyLight 650 NHS Ester, Colorada 645 XT A -

NHS ester, Fluorescentred 647 reactive, CF647 Succinimidyl ester and PromoFluor-647, NHS ester for the required	
applications.	

applications.
Near Infrared Fluorophore Spectral Data & Quencher Selection Guide
Fluorophore Name
Absorbance Max, nm +/-10
Emission Max, nm +/-10
Extinction Coefficient*
Color**
Quencher
Cy5 650 665 250,000
IRDye 650 NHS 650 665 230,000
AZ647 NHS 655 680 191,800

AZ680 NHS 678 701 185,000



Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000 IRDye 750 NHS 756 776 260,000



cy7.5 NHS 788 808 223,000

IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), 4: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), 28: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), 14: 303-308.
- 4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), 76: 922-926.

Reaction scheme for primary amine labelled oligos with NHS ester is shown in the figure below.

-





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Cy5 Internal

#### Click here for a list of fluorophores.

Cyanine 5 (Cy5) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy5 is reactive, water-soluble, and has an absorbance maximum of 649 nm and an emission maximum of 670 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy5 is most commonly paired with the dark quencher BHQ-3, as the two have excellent spectral overlap.

Cy5 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy5 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy5-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient\*

Color\*\*

Quencher



genelink.com/newsite/products/mod\_detail.asp?modid=27">Cy5 650 665 250,000 IRDye 650 NHS 650 665 230,000 AZ647 NHS 655 680 191,800 AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000



Atto 725 NHS 728 751 120,000
Atto 740 NHS 743 763 120,000
Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
$^*$ Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.
Click here for a list of fluorophores.
Click here for list of quenchers.  References  1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite and provide a quenched probe system useful for detecting PCP product and public acid hybridization PCP.



Methods Appl. (1995), 4: 1-6.

- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.
- 4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), **76**: 922-926.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Cy5 NHS

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Cy5 can be used as a replacement for Alexa Fluor 647 Succinimidyl Ester, DyLight 650 NHS Ester, Colorada 645 XT A - NHS ester, Fluorescent red 647 reactive, CF647 succinimidyl ester and PromoFluor-647 NHS ester for the required applications.

Cyanine 5 (Cy5) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy5 is reactive, water-soluble, and has an absorbance maximum of 649 nm and an emission maximum of 670 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy5 is most commonly paired with the dark quencher BHQ-3, as the two have excellent spectral overlap.

Cy5 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy5 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy5-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

**Extinction Coefficient\*** 

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000



AZ647 NHS 655 680 191,800 AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000



Cy7 NHS 740 773 199,000	
IRDye 750 NHS 756 776 260,000	
cy7.5 NHS 788 808 223,000	
IRDye 800 NHS 795 819 240,000	

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), 4: 1-6.
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- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), 14: 303-308.
- 4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), 76: 922-926.

Reaction scheme for primary amine labelled oligos with NHS ester is shown in the figure below.







Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Cy5-3'

Category Fluorescent Dyes Cy5-3 Modification Code Reference Catalog Number 26-6549 5 Prime Ν 3 Prime Υ Internal Ν www.Oligo-5 Molecular Weight(mw) 533.63 3'-Cy5 Label OH [26-6549]

### Click here for a list of fluorophores.

Cyanine 5 (Cy5) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy5 is reactive, water-soluble, and has an absorbance maximum of 649 nm and an emission maximum of 670 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5'or 3' end, or internally. Cy5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy5 is most commonly paired with the dark quencher BHQ-3, as the two have excellent spectral overlap.

Cy5 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy5 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy5-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient\*

Color\*\*

Quencher



genelink.com/newsite/products/mod\_detail.asp?modid=27">Cy5 650 665 250,000 IRDye 650 NHS 650 665 230,000 AZ647 NHS 655 680 191,800 AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000



Atto 725 NHS 728 751 120,000
Atto 740 NHS 743 763 120,000
Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
$^*$ Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.
Click here for a list of fluorophores.
Click here for list of quenchers.  References  1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization PCR



Methods Appl. (1995), 4: 1-6.

- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
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Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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### Cy5-5'

Category Fluorescent Dyes

Modification Code Cy5-5

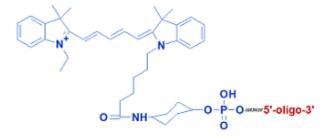
Reference Catalog Number 26-6436

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 656.81



Cy5-5' [26-6436-XX]

#### Click here for a list of fluorophores.

Prices listed above are for 5' modification. Internal and 3' incurs additional charges and are post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Cyanine 5 (Cy5) is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy5 is reactive, water-soluble, and has an absorbance maximum of 649 nm and an emission maximum of 670 nm. It is available as both a phosphoramidite and an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy5 is most commonly paired with the dark quencher BHQ-3, as the two have excellent spectral overlap.

Cy5 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy5 double-labeling of FISH probes (at both ends) that were specific to ribsosomal RNA targets in microorganisms at least doubles FISH signal intensity without affecting specificity. This Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) strategy may provide an effective solution to the problem of low signal intensity, which is commonly observed when using corresponding singly-labeled FISH probes for microbe identification. As an added benefit, Cy5-doubly labeled probes were shown to increase the in situ accessibility of rRNA targets sites in microbes, which allows for greater probe design flexibility.

Click here for list of quenchers.

Click here for a list of fluorophores.



Near Infrared Fluorophore Spectral Data & Quencher Selection Guide
Fluorophore Name
Absorbance Max, nm +/-10
Emission Max, nm +/-10
Extinction Coefficient*
Color**
Quencher
Cy5 650 665 250,000
IRDye 650 NHS 650 665 230,000
AZ647 NHS 655 680 191,800
AZ680 NHS 678 701 185,000
Cy5.5 684 710 198,000
IRDye 700 NHS 684 710 288,000



AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000 IRDye 750 NHS 756 776 260,000 cy7.5 NHS 788 808 223,000



 $^*$  Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers. References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), 4: 1-6.
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- 4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), 76: 922-926.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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### Cy5.5

Category Fluorescent Dyes Cy5.5-5 Modification Code Reference Catalog Number 26-6460 5 Prime Υ 3 Prime Ν Internal Ν ~~~Oliao-3' 5'-Cy5.5 Label Molecular Weight(mw) 633.74 [26-6460-XX] OH

Cyanine 5.5 (Cy5.5) is a far-red/near infra red (NIR) fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy5.5 is reactive, water-soluble, and has an absorbance maximum of 675 nm and an emission maximum of 694 nm. It is available as a phosphoramidite, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy5.5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy5.5 is most commonly paired with the dark quencher BHQ-3, as the two have excellent spectral overlap.

Cy5.5 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH).

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient\*

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000



asp?modid=516">AZ647 NHS 655 680 191,800 AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000



Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
* Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for list of quenchers.

Click here for a list of fluorophores.

References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), 4: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), 28: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), 14: 303-308.
- 4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), 76: 922-926.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Cy5.5-Internal

Category Fluorescent Dyes Cy5.5-Int Modification Code Reference Catalog Number 26-64601 5 Prime 3 Prime Υ Internal Υ ~~~Oliao-3' 5'-Cy5.5 Label Molecular Weight(mw) 633.74 [26-6460-XX] OH

Cyanine 5.5 (Cy5.5) is a far-red/near infra red (NIR) fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy5.5 is reactive, water-soluble, and has an absorbance maximum of 675 nm and an emission maximum of 694 nm. It is available as a phosphoramidite, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Cy5.5 plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, Cy5.5 is most commonly paired with the dark quencher BHQ-3, as the two have excellent spectral overlap.

Cy5.5 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH).

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient\*

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000



asp?modid=516">AZ647 NHS 655 680 191,800 AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000



Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
$^*$ Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers. References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), 4: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), 28: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), 14: 303-308.
- 4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), 76: 922-926.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Cy7 NHS

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne: Azide, Azide: DBCO, BCN: Azide, BCN: TCO: Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide Fluorophore Name Absorbance Max, nm +/-10 Emission Max, nm +/-10 **Extinction Coefficient\*** Color\*\* Quencher Cy5 650 665 250,000 IRDye 650 NHS 650 665 230,000 AZ647 NHS 655 680 191,800 AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000



IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000 IRDye 750 NHS 756 776 260,000 cy7.5 NHS 788 808 223,000



#### IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

Cyanine 7(Cy7) NHS ester is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy7 is reactive, water-soluble, and has an absorbance maximum of 747 nm and an emission maximum of 776 nm, which is in the near IR. It is available as an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. Because it is a near IR dye, Cy7 has very little background fluorescence associated with it (1). It is thus an excellent choice for labeling oligo probes slated for in vivo applications, because the minimal scattering and absorption of near-IR photons by cellular tissue ensures higher S/N ratio, and better sensitivity. For example, Fluorescent Resonance Energy Transfer (FRET) oligonucleotide duplexes using Cy5.5 as the donor on one strand and Cy7 as the acceptor on the complementary strand have been used to detect and characterize transcription factor NF-kappaB p50 protein binding to DNA (2)

Caution: Cy7 is intensely colored and very reactive. Care should be exercised when handling the vial containg the C7-labeled oligo to avoid staining clothing, skin, and other items. Also, because Cy7 is in the form of an NHS ester, the oligo first must be synthesized with an Amino C6 Linker (for the ends) or the Amino C6 version of the base phosphoramidite (for internal labeling). The Cy7-NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis.

References

- 1. Benson, R.C., Kues, H.A. Absorption and Fluorescence Properties of Cyanine Dyes. *J. Chem. Eng. Data* (1977), 22: 379-383.
- 2. Zhang, S., Metelev, V., Tabatadze, D., Zamecnik, P.C., Bogdanov, A. Fluorescence resonance energy transfer in near-infrared fluorescent oligonucleotide probes for detecting protein-DNA interactions. *Proc. Nat. Acad. Sci. USA.* (2008), 105: 4156-4161.





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### Oligo Modifications

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### **Cy7.5 NHS**

Category Fluorescent Dyes

Modification Code Cy7.5-N

Reference Catalog Number 26-6498

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 664.91

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne: Azide, Azide: DBCO, BCN: Azide, BCN: TCO: Tetrazine. Gene Link offers a wide selection of click modifications for 5'. 3' and internal sites. Click here for a list of click chemistry modifications.

Cyanine 7.5 (Cy7.5) NHS ester is a fluorescent dye that belongs to the Cyanine family of synthetic polymethine dyes. Cy7.5 is reactive, water-soluble, and has an absorbance maximum of 788 nm and an emission maximum of 808 nm, which is in the near IR. It is available as an NHS ester, and is used to fluorescently label oligonucleotides at either the 5' or 3' end, or internally. As a near IR dye, Cy7.5 has very little background fluorescence associated with it (1). It is thus an excellent choice for labeling oligo probes slated for in vivo applications, because the minimal scattering and absorption of near-IR photons by cellular tissue ensures higher S/N ratio, and better sensitivity. For example, Fluorescent Resonance Energy Transfer (FRET) oligonucleotide duplexes using Cy5.5 as the donor on one strand and Cy7.5 as the acceptor on the complementary strand have been used to detect and characterize transcription factor NF-kappaB p50 protein binding to DNA (2)

Caution: Cy7.5 is intensely colored and very reactive. Care should be exercised when handling the vial containing the C7.5-labeled oligo to avoid staining clothing, skin, and other items. Also, because Cy7.5 is in the form of an NHS ester, the oligo first must be synthesized with an Amino C6 Linker (for the ends) or the Amino C6 version of the base phosphoramidite

(for internal labeling). The Cy7.5-NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis.	.6
Near Infrared Fluorophore Spectral Data & Quencher Selection Guide	

Absorbance Max, nm +/-10

Fluorophore Name

Emission Max, nm +/-10

Extinction Coefficient\*

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800



AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000



Cy7 NHS 740 773 199,000
IRDye 750 NHS 756 776 260,000
cy7.5 NHS 788 808 223,000
IRDye 800 NHS 795 819 240,000
* Extinction coefficient at $\lambda$ (max) in cm-1M-1. ** Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.
Click here for a list of fluorophores.

Click here for list of quenchers.

References

- 1. Benson, R.C., Kues, H.A. Absorption and Fluorescence Properties of Cyanine Dyes. *J. Chem. Eng. Data* (1977), 22: 379-383.
- 2. Zhang, S., Metelev, V., Tabatadze, D., Zamecnik, P.C., Bogdanov, A. Fluorescence resonance energy transfer in near-infrared fluorescent oligonucleotide probes for detecting protein-DNA interactions. *Proc. Nat. Acad. Sci. USA.* (2008), 105: 4156-4161.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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### **Dansyl-X**

Category	Fluorescent Dyes	H <sub>3</sub> C CH <sub>3</sub>	
Modification Code	Dnsl-X	N	
Reference Catalog Number	26-6458		Dansyl-X Oligo
5 Prime	Υ		[26-6458-XX]
3 Prime	Υ	W Y	[20-0400-7/7]
Internal	Υ	o=\$=0	o I
Molecular Weight(mw)	461.53	ŃН	// Mm Oligo

Dansyl-X modification is a post synthesis conjugation to a primary amino group. The amino group can be placed at the 5' and 3' and for internal positions an amino modified base is used, e.g Amino dT C6 Dansyl-X NHS dye is a post synthesis conjugation dye requiring an amino group on the oligo. The amino group can be placed at the 5' or 3' or any internal position using amino C6 base. Ideally we recommend to use amino C6- dT or U for RNA oligo.

Dansyl dyes have environmentally sensitive fluorescence quantum yields and emission maxima along with large Stokes shifts. This environment-sensitive fluorescence property has made Dansyl dyes an important tool for biophysical studies. They are particularly useful for preparing fluorescent drug or ligand analogs that are expected to bind to hydrophobic sites in proteins or membranes or other biological receptors. Dansyl protein conjugates have fluorescence lifetimes of 10-20 nanoseconds.





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### Oligo Modifications

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### **DNP TEG (2, 4-dinitrophenyl)**

Category Affinity Ligands

Modification Code DNP-TEG

Reference Catalog Number 26-6512

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 509.41

DNP (2,4-dinitrophenyl) is classified as a hapten for molecular biology purposes, that is, a small molecule having high immunogenicity. Because antibodies raised against haptens have considerably higher affinities for them than other antibodies do for their targets makes haptens particularly desirable as affinity tags for oligonucleotides (1).

DNP attached to a triethylene glycol (TEG) spacer arm is commonly used to label oligonucleotides probes for use in hybridization applications, for example, in situ hybridization, Northern and Southern blotting (2). After hybridization to their targets, these DNP-labeled probes are detected with anti-DNP antibodies that are labeled with dyes (for primary detection) or enzymes (for secondary detection using a fluorogenic, chemiluminogenic, or colorimetric (3) substrate specific for the enzyme). To maximize signal obtained with such probes, Gene Link recommends modifying the oligonucleotide probe with three DNP molecules, either grouped at the 5'-end or spaced about 10 bases apart (2).

In addition to the above straightforward anti-DNP antibody-based detection systems, oligo probes labeled with both a fluorescent dye and DNP also been used for highly-sensitive direct detection of antigens (at femtoMolar levels) in a rolling circle amplification (RCA)-based assay system (4). **References** 

- 1. Shreder, K. Synthetic Haptens as Probes of Antibody Response and Immunorecognition. *Methods (Academic Press)* (2000), **20**: 372-379.
- 2. Grzybowski, J., Will, D.W., Randall, R.E., Smith, C.A., Brown, T.. Synthesis and antibody-mediated detection of oligonucleotides containing multiple 2,4-dinitrophenyl reporter groups. *Nucleic Acids Res.* (1993), **21**: 1705-1712.

  3. Lehtovaara, P., Uusi-Oukari, M., Buchert, P., Laaksonen, M., Bengtstrom, M. Ranki, M. Quantitative PCR for Hepatitis B
- 3. Lentovaara, P., Uusi-Oukari, M., Buchert, P., Laaksonen, M., Bengtstrom, M. Ranki, M. Quantitative PCR for Hepatitis E Virus with Colorimetric Detection. *Genome Res.* (1993), **3**: 169-175.
- 4. Schweitzer, B., Wiltshire, S., Lambert, J., O'Malley, S., et al. Immunoassays with rolling circle DNA amplification: A versatile platform for ultrasensitive antigen detection. *Proc. Natl. Acad. Sci. (USA)* (2000), **97**: 10113-10119.





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N —

### Oligo Modifications

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### etheno dA

		$N \longrightarrow N$
Category	Minor Bases	5' Oligo
Modification Code	Eth-dA	0=P-0-1 N N
Reference Catalog Number	26-6506	он 🗸
5 Prime	Υ	
3 Prime	Υ	0=P-0-/// Oligo 3'
Internal	Υ	OH
Molecular Weight(mw)	337.23	etheno deoxyadenosine dA [26-6506-XX]

1,N-6 etheno deoxyadenosine (Etheno-dA) is a highly fluorescent derivative of dA, and can be incorporated at any position(s) within a DNA or RNA oligonucleotide. Etheno-dA has excitation maxima at 270 nm and 300 nm, and an emission maximum at 410 nm. Selective introduction of etheno-dA into DNA or RNA oligonucleotides is particularly useful in various structure-function studies of RNA, protein-RNA complexes, and DNA-RNA based diagnostics applications (1). However, because etheno-dA does not base-pair with dT or dU, oligos containing etheno-dA at either the 3'-end or in the middle will not function as either a sequencing or PCR primer. Etheno-dA-modified primers must have the modification(s) located either at or close to the 5'-end in order to so function (1).

Etheno-dA-modified oligonucleotides have proven particularly useful in the study of the repair of alkylated DNA damage by the base-excision-repair (BER) mechanism For example, such modified oligos were used to elucidate the function of N-methylpurine DNA glycosylase (2), as well as providing insights into how this BER enzyme facilitates resistance of astrocyte brain tumors (malignant astrocytomas) to DNA-alkylation-based chemotherapy agents (such as nitrosoureas) (3). Exocyclic etheno DNA adducts likely play an important role in carcinogenesis in both rodents and humans (4), and etheno-dA-modified oligonucleotides can be used as research tools for the study of carcinogenesis in various tissues. **References** 

- 1. Srivastava, S.C., Raza, S.K., Misra, R. 1,N6-etheno deoxy and ribo adenoGine and 3,N4-etheno deoxy and ribo cytidine phosphoramidites. Strongly fluorescent structures for selective introduction in defined sequence DNA and RNA molecules. *Nucleic Acids Res.* (1994), **22**: 1296-1304.
- 2. Dosanjh, M.K., Roy, R., Mitra, S., Singer, B. 1, N6-ethenoadenine is preferred over 3-methyladenine as substrate by a cloned human N-methylpurine-DNA glycosylase (3-methyladenine-DNA glycosylase). *Biochemistry* (1994), **33**: 61624-1628. 3. Harrison, J.F., Rinne, M.L., Kelley, M.R., Druzhyna, N.M., Wilson, G.L., Ledoux, S.P. Altering DNA Base Excision Repair: Use of Nuclear and Mitochondrial-Targeted N-Methylpurine DNA Glycosylase to Sensitize Astroglia to Chemotherapeutic Agents. *Glia*. (2007), **55**: 1416-1425.
- 4. Chung, F-L., Chen, H-J.C., Nath, R.G. Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts.



Carcinogenesis (1996), 17: 2105-2111.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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### Fam (6-FAM)-3'

Category Fluorescent Dyes

Modification Code Fam-3

Reference Catalog Number 26-6516

5 Prime N

3 Prime Y

Internal N

Molecular Weight(mw) 569.45 6-Fam-3' (6-fluorescein amidite-3')

[26-6516-XX]

6-carboxyfluorescein (6-FAM) is the most commonly used fluorescent dye for labeling oligonucleotides. 6-FAM is reactive, water-soluble, and has an absorbance maximum of 492 nm and an emission maximum of 517 nm. 6-FAM plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, 6-FAM is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap. 6-FAM-labeled primers have also been used for bacterial SNP genotyping by allele-specific real-time PCR (4).

- 6-FAM can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with 6-FAM at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References**
- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995) 4: 1-6
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.
- 4. Huygens, F., Inman-Bamber, J., Nimmo-G.R., Munckhof, W., Schooneveldt, J., Harrison, B., McMahon, J.A., Giffard, P.M. Staphylococcus aureus Genotyping Using Novel Real-Time PCR Formats. *J. Clin. Microbiol.* (2006), **44**: 3712-3718.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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### Fam (6-FAM)-5'

Category Fluorescent Dyes

Modification Code Fam-5

Reference Catalog Number 26-6431

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 567.6

NH NH Owwoligo -3'

Fam (6-fluorescein amidite (6-FAM))-5' [26-6431-XX]

#### Click here for a list of fluorophores.

6-carboxyfluorescein (6-FAM) is the most commonly used fluorescent dye for labeling oligonucleotides. 6-FAM is reactive, water-soluble, and has an absorbance maximum of 492 nm and an emission maximum of 517 nm. 6-FAM plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, 6-FAM is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap. 6-FAM-labeled primers have also been used for bacterial SNP genotyping by allele-specific real-time PCR (4).

- 6-FAM can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with 6-FAM at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References**
- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.
- 4. Huygens, F., Inman-Bamber, J., Nimmo-G.R., Munckhof, W., Schooneveldt, J., Harrison, B., McMahon, J.A., Giffard, P.M. Staphylococcus aureus Genotyping Using Novel Real-Time PCR Formats. *J. Clin. Microbiol.* (2006), **44**: 3712-3718.





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# Oligo Modifications

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### Fam (6-FAM)-Internal

Category Fluorescent Dyes

Modification Code Fam-Int

Reference Catalog Number 26-6431I

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 567.6 Fam (6-fluorescein amidite (6-FAM) Internal

[26-6431I-XX]

5'-Oligo wwwo

0.4444VOligo -3'

#### Click here for a list of fluorophores.

6-carboxyfluorescein (6-FAM) is the most commonly used fluorescent dye for labeling oligonucleotides. 6-FAM is reactive, water-soluble, and has an absorbance maximum of 492 nm and an emission maximum of 517 nm. 6-FAM plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, 6-FAM is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap. 6-FAM-labeled primers have also been used for bacterial SNP genotyping by allele-specific real-time PCR (4).

- 6-FAM can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with 6-FAM at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References**
- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.

  2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation
- detection. Nucleic Acids Res. (2000), 28: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.
- 4. Huygens, F., Inman-Bamber, J., Nimmo-G.R., Munckhof, W., Schooneveldt, J., Harrison, B., McMahon, J.A., Giffard, P.M. Staphylococcus aureus Genotyping Using Novel Real-Time PCR Formats. *J. Clin. Microbiol.* (2006), **44**: 3712-3718.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Fam dT

Fluorescein-dT is a deoxythymidine nucleoside derivitized with 6-FAM (6-carboxyfluorescein) through a spacer arm. 6-FAM is the most commonly used fluorescent dye for labeling oligonucleotides; Fluorescein-dT is used to internally label an oligonucleotide at a dT position. Fluorescein-dT has an absorbance maximum of 492 nm and an emission maximum of 517 nm. Fluorescein-dT can be used to internally label a Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a fluorophore. Such a labeling strategy is pertinent in cases where the distance between the quencher and fluorophore needs optimization for efficient quenching. For such probes, fluorescein is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap.

Fluorescein-dT also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos internally labeled with fluorescein-dT also can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.



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# Oligo Modifications

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#### **Fam Serinol**

Category Fluorescent Dyes Modification Code Fam-Ser Reference Catalog Number 26-6503 5 Prime 3 Prime Υ Internal Υ 0 6-FAM Serinol Molecular Weight(mw) 582.45 [26-6503-XX] ÓН

#### Click here for a list of fluorophores.

Fam serinol is 6-carboxyfluorescein (6-FAM) Serinol. This can be used as a non-nucleosidic modification for internal labeling and will be inserted in the backbone of the oligo and can be extended.

6-carboxyfluorescein (6-FAM) is the most commonly used fluorescent dye for labeling oligonucleotides. 6-FAM is reactive, water-soluble, and has an absorbance maximum of 492 nm and an emission maximum of 517 nm. 6-FAM plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, 6-FAM is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap. 6-FAM-labeled primers have also been used for bacterial SNP genotyping by allele-specific real-time PCR (4).

- 6-FAM can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with 6-FAM at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References**
- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308. 4. Huygens, F., Inman-Bamber, J., Nimmo-G.R., Munckhof, W., Schooneveldt, J., Harrison, B., McMahon, J.A., Giffard, P.M.
- Staphylococcus aureus Genotyping Using Novel Real-Time PCR Formats. *J. Clin. Microbiol.* (2006), **44**: 3712-3718.



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### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Fam-NHS (6-Fam NHS)

Category	Fluorescent Dyes		
Modification Code	Fam-N		CH <sub>3</sub> CH <sub>3</sub>
Reference Catalog Number	26-6730	Fam NHS	
5 Prime	Υ	[26-6730-XX]	<b>o</b>
3 Prime	Υ		<b>\( \)</b>
Internal	Υ	Amino Linker	HN
Molecular Weight(mw)	358		Oligo

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

6-carboxyfluorescein (6-FAM) is the most commonly used fluorescent dye for labeling oligonucleotides. 6-FAM is reactive, water-soluble, and has an absorbance maximum of 492 nm and an emission maximum of 517 nm. 6-FAM plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, 6-FAM is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap. 6-FAM-labeled primers have also been used for bacterial SNP genotyping by allele-specific real-time PCR (4).

6-FAM can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with 6-FAM at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

#### References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.
- 4. Huygens, F., Inman-Bamber, J., Nimmo-G.R., Munckhof, W., Schooneveldt, J., Harrison, B., McMahon, J.A., Giffard, P.M. Staphylococcus aureus Genotyping Using Novel Real-Time PCR Formats. *J. Clin. Microbiol.* (2006), **44**: 3712-3718.





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# Oligo Modifications

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#### Fam-TEG Azide

This modification is a post synthesis conjugation to an alkyne or DBCO modification at the appropriate site for click conjugation.

6-FAM (6-carboxyfluorescein)-TEG Azide is a 6-FAM fluorescent dye attached to a 15-atom mixed polarity triethylene glycol spacer with an azide group at the end of the spacer. 6-FAM is the most commonly used fluorescent dye for labeling oligonucleotides, and is reactive and water-soluble, with an absorbance maximum of 492 nm and an emission maximum of 517 nm. The presence of the azide allows the user to use "Click Chemistry" (a [3+2] cycloaddition reaction between alkynes and azides, using copper (I) iodide as a catalyst) to conjugate the 6-FAM-TEG Azide to a terminal alkyne-modified oligo with extremely high regioselectivity and efficiency (1,2). Preparation of the alkyne-modified oligo can be achieved using the 5'-Hexynyl modifier (see its respective tech sheet for details). The spacer acts to minimize steric hindrance between the biotin moiety and the oligo. **References** 

- 1. Huisgen, R. Angew. Chem. Int. Ed. (1963), 2: 565-568.
- 2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.



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# Oligo Modifications

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#### Ferrocene-dT

Category Redox Electrochemical Modification Code Fc-dT

26-6906 Reference Catalog Number

5 Prime

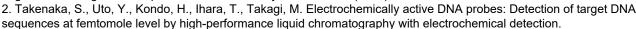
3 Prime

Internal Υ

694.53 Molecular Weight(mw)

Ferrocene oligonucleotides should be stored under Argon and aqueous solutions should be degassed immediately. A convenient way to degas is the use of vacuum desiccator. We suggest making multiple small aliquots for storage at -20C or -80C for long term storage.

Ferrocene-dT is a modified base nucleotide that contains a redox-active ferrocene moiety. Ferrocene is a sandwich compound composed of two cyclopentadienyl rings bound on opposite sides of a central iron atom (1). When incorporated into an oligonucleotide, the presence of ferrocene enables its use as an electrochemical (EC) probe for nucleic acid analysis. Ferrocene-modified probes can be designed to bind to either single- or double-stranded targets, and the resulting double- or triple-stranded probe-target complex is typically detected by HPLC with a standard electrochemical detector, with reported sensitivity at the sub-femtomole level (2,3). Ferrocence-modified probes covalently attached to a gold electrode surface have also been used in EC-based SNP assay, one probe to detect wild-type, and the other the SNP (4). In an alternative format, a "sandwich SNP assay" has also been studied. Here, a capture oligo was covalently bound to a gold surface via several phosphorothiolate linkages to capture the desired target DNA and hold it close to the gold surface. The targeted region for the capture oligo contains the SNP. A second, ferrocene-modified detection probe, hybridizes to a different, highly conserved, part of the target oligo to serve as the detector. If the target has been captured, electron transfer occurs between the ferrocene of the detection probe and the gold surface, producing an electrochemical signal (5). Ferrocene-modified DNA aptamers, designed to bind to one specific biochemical target molecule (DNA, RNA, proteins, etc.) have also been used to make aptamer-based EC sensors (6). EC probes also have significant potential as a low cost alternative to fluorescent-based probes in DNA microarray systems designed for use in clinical or medical diagnosis (7,8). References 1. Neto, F., Pelegrino, A., Caramori, A., Darin, V.A. Ferrocene: 50 Years of Transition Metal Organometallic Chemistry—From Organic and Inorganic to Supramolecular Chemistry. ChemInform (2004), 35: no. doi: 10.1002/chin.200443242. 2. Takenaka, S., Uto, Y., Kondo, H., Ihara, T., Takagi, M. Electrochemically active DNA probes: Detection of target DNA





- Anal. Biochem. (1994), 218: 436-443.
- 3. Ihara, T., Maruo, Y., Takenaka, S., Takagi, M. Ferrocene-oligonucleotide conjugates for electrochemical probing of DNA. *Nucleic Acids Res.* (1996), **24**: 4273-4280.
- 4. Yu, C.J., Wan, Y., Yowanto, H., Li, J., Tao, C., James, M.D., Tan, C.L., Blackburn, G.F., Meade, T.J. Electronic Detection of Single-Base Mismatches in DNA with Ferrocene-Modified Probes. *J. Am. Chem. Soc.* (2001), **123**: 11155-11161. 5. Nakayama, N., Ihara, T., Nakano, K., Maeda, M. DNA sensors using a ferrocene-oligonucleotide conjugate. *Talanta* (2002), **56**: 857-866.
- 6. Radi, A-E., Sanchez, J.L.A., Baldrich, E., O'Sullivan, C.K. Reagentless, Reusable, Ultrasensitive Electrochemical Molecular Beacon Aptasensor. *J. Am. Chem. Soc.* (2006), **128**: 117-124.
- 7. Liepold, P., Wieder, H., Hillebrandt, H., Friebel, A., Hartwich, G. DNA-arrays with electrical detection: A label-free low cost technology for routine use in life sciences and diagnostics. *Bioelectrochem.* (2005), **67**: 143-150.
- 8. Liepold, P., Kratzmuller, T., Persike, N., Bandilla, M., Hinz, M., Wieder, H., Hillebrandt, H., Ferrer, E., Hartwich, G. Electrically detected displacement assay (EDDA): a practical approach to nucleic acid testing in clinical or medical diagnosis. *Anal. Bioanal. Chem.* (2008), **391**: 1759-1772.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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#### Fluorescein-3'

Category Fluorescent Dyes

Modification Code FI-3

Reference Catalog Number 26-6595

5 Prime N

3 Prime Y

Internal N

Molecular Weight(mw) 569.45 Fluorescein-3' (FITC (Fluorescein isothiocyanate)-3')

[26-6595-XX]

Fluorescein is the most commonly used fluorescent dye for labeling oligonucleotides and is also known as FITC (Fluorescein isothiocyanate). Fluorescein has an absorbance maximum of 494 nm and an emission maximum of 521 nm. The difference between the fluorescein and 6-FAM modifications is that here the fluorescein dye is attached to the 6-carbon spacer via an N-hydroxysuccinimide (NHS) group instead of a carboxy group. Fluorescein plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, fluorescein is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap.

Fluorescein can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with fluorescein at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References** 

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





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## Oligo Modifications

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#### Fluorescein-5'

Category

Fluorescent Dyes

Modification Code

FI-5

Reference Catalog Number

5 Prime

Y

3 Prime

N

Internal

Molecular Weight(mw)

Fluorescein isothiocyanate) 5'

[26-6426-XX]

Fluorescein is the most commonly used fluorescent dye for labeling oligonucleotides and is also known as FITC (Fluorescein isothiocyanate). Fluorescein has an absorbance maximum of 494 nm and an emission maximum of 521 nm. The difference between the fluorescein and 6-FAM modifications is that the fluorescein dye is attached to the 6-carbon spacer via an N-hydroxysuccinimide (NHS) group instead of a carboxy group. Fluorescein plays a particularly important role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, fluorescein is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap.

Fluorescein can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with fluorescein at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References** 

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





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# Oligo Modifications

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#### Hex-5'

Category Fluorescent Dyes

Modification Code Hex-5

Reference Catalog Number 26-6432

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 744.13

5' Hexachloro-Fluorescein (HEX) [26-6432-XX]

#### Click here for a list of fluorophores.

Hexachloro-fluorescein (HEX) is hexachlorinated version of the fluorescent dye fluorescein, and is used for labeling oligonucleotides at either the 5' or 3' end. HEX has an absorbance maximum of 535 nm and an emission maximum of 556 nm. HEX can be used in real-time PCR applications as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, HEX is most commonly paired with the dark quencher BHQ-1, as the two have good spectral overlap.

HEX also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with HEX at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. NOTE: If HEX is on the 3' end of the oligo, it cannot be used as a primer in PCR-based applications.

Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.



5 Red 588 604 ROX Red 575 602 Texas Red Red 583 603

#### Click here for a list of fluorophores.

#### References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





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### Oligo Modifications

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#### Hex-Azide-6

CI HO OH Category Click Chemistry Modification Code Hex-N3 CI CI CI Reference Catalog Number 26-6723 5 Prime 3 Prime CI HN Internal Υ 6-HEX Azide Molecular Weight(mw) 665.09 [26-6723-XX]

This modification is a post synthesis conjugation to an alkyne or DBCO modification at the appropriate site for click conjugation.

HEX (Hexachloro-fluorescein)-Azide is a fluorescent dye containing an terminal azide group. HEX has an absorbance maximum of 535 nm and an emission maximum of 556 nm. The presence of the azide allows the user to use "Click Chemistry" (a [3+2] cycloaddition reaction between alkynes and azides, using copper (I) iodide as a catalyst) to conjugate the HEX-Azide to a terminal alkyne-modified oligo with extremely high regioselectivity and efficiency (1,2). Preparation of the alkyne-modified oligo can be achieved using the 5'-Hexynyl (Alkyne) modifier or for copper free conjugation use the cyclooctyne DBCO dT, DBCO TEG(see its respective tech sheet for details). **References**1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2**: 565-568.

2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.





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## Oligo Modifications

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### **HEX-N**

Category	Fluorescent Dyes	
Modification Code	Hex-N	CI CI HO O OH
Reference Catalog Number	26-6590	
5 Prime	Υ	CÍ CI O CI
3 Prime	Υ	O HN CI
Internal	Υ	0     0
Molecular Weight(mw)	744.13	5' Hexachloro-Fluorescein (HEX)

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Hexachloro-fluorescein (HEX) is hexachlorinated version of the fluorescent dye fluorescein, and is used for labeling oligonucleotides at either the 5' or 3' end. HEX has an absorbance maximum of 535 nm and an emission maximum of 556 nm. HEX can be used in real-time PCR applications as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, HEX is most commonly paired with the dark quencher BHQ-1, as the two have good spectral overlap.

HEX also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with HEX at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. NOTE: If HEX is on the 3' end of the oligo, it cannot be used as a primer in PCR-based applications. **References** 

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **HEX-SIMA dT**

### Click here for a list of fluorophores.

HEX-SIMA is Dichloro-diphenyl-fluorescein and exhibits virtually identical absorbance and emission spectra to HEX. HEX-SIMA dT can be placed at any site of an oligo. This is specially suited for internal positions. Hexachloro-fluorescein (HEX) is hexachlorinated version of the fluorescent dye fluorescein, and is used for labeling oligonucleotides at either the 5' or 3' end. HEX has an absorbance maximum of 535 nm and an emission maximum of 556 nm. HEX can be used in real-time PCR applications as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, HEX is most commonly paired with the dark quencher BHQ-1, as the two have good spectral overlap.

HEX-HEX also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with HEX at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.



5 Red 588 604 ROX Red 575 602 Texas Red Red 583 603

#### Click here for a list of fluorophores.

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Hyper5

Category Fluorescent Dyes

Modification Code Hyper5

Reference Catalog Number 26-6705

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 898 proprietary to GE Healthcare and its chemical structure currently is not

publicly available.

fluorescent

dye

ls

**YIELD** Hyper5 dye conjugation to oligos is performed post synthesis using NHS to amine reaction and thus the yield obtained is lower than other chemically modified oligos.

Hy per5

~500 pmole (0.5nmol) final yield for 200 nmol scale

~1 nmole final yield for 1 umol scale

HyPer5 is a red fluorescent dye that is spectrally similar to Cy5, Cy5.5 and Alexa 660 (HyPer5 has an absorbance maximum of 664 nm and an emission maximum of 680 nm), but is significantly more resistant to degradation from both light and ozone exposure than Cy5 (or other commercial dyes). HyPer5 was specifically developed as an alternative to labeling oligonucleotides slated for use as probes in microarray experiments with Cy5. Cy5 (and Alexa 647) is known to rapidly degrade during the summer, when atmospheric ozone concentrations reach 25 parts per billion (ppb) (1). In addition, Cy5 is sensitive to photo-bleaching, which can lead to significant distortion of the dye ratios (for example Cy5/Cy3) used in copy number analysis of microarray results, even under low ozone conditions (for example, during winter). HyPer5 was developed to address both of these problems; it is highly resistant to degradation at ozone levels as high as 300 ppb (10X higher than that observed in summer) and 3-4X more photostable than Cy5 (2). These improved properties of HyPer5 over Cy5 make it an attractive option for anyone performing two-color microarray experiments.

Note that because HyPer5 is in the form of an NHS ester, an amino linker (such as Amino Linker C6) moiety must first be incorporated into the oligonucleotide in order to place an active primary amino group at the desired position (either at the end or internally). HyPer5-NHS is then conjugated to the amino group in a separate reaction to form the final HyPer5-labeled product and the yield is low.

If Hyper 5 (Emission=680 nm & Absorbance=664 nm) specific properties of ozone and photo-bleaching resistance is not critical and higher dye label yields are desired then as substitutes we suggest Cy5.5 (Emission=707 nm & Absorbance=683 nm) or Alexa 660 (Emission=690 nm & Absorbance=663 nm) that has emission and absorbance in the same range.

- 1. Fare, T.L., Coffey, E.M., Dai, H., He, Y.D., et al. Effects of atmospheric ozone on microarray data quality. *Anal. Chem.* (2003), **75**: 4672-4675.
- 2. Dar, M., Giesler, T., Richardson, R., Cai, Christine, Cooper, M.



, Lavasani, S., Kille, P., Voet, T., Vermeesch, J. Development of novel ozone- and photo-stable HyPer5 red fluorescent dye for array CGH and microarray gene expression analysis with consistent performance irrespective of environmental conditions. *BMC Biotechnol.* (2008), **8**: 86.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

dve

ls

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### IRDye 650LT-N

Category Fluorescent Dyes

Modification Code IRD650LT-N

Reference Catalog Number 26-6646

Υ 5 Prime

3 Prime Υ

Internal Υ

fluorescent proprietary to GE Healthcare and its Molecular Weight(mw) 905 chemical structure currently is not

publicly available.

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Hy per5

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. NHS Ligand conjugation requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Maleimide Ligand conjugation requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

IR650LT-N is a near-IR fluorescent dye used for labeling oligonucleotides. IR650LT-N has an absorbance maximum of 672 nm and an emission maximum of 695 nm. The combination of narrow absorbance/emission bands and low-background autofluorescence in the IR region results in higher S/N ratios and thus enhanced detection sensitivity compared with fluorophores with absorbance/emission maxima in the visible region (1). IRDye700 is used as a reporter moiety in real-time PCR applications. For such probes, IRDye700 is most commonly paired with the dark quencher BBQ-650, as the two have excellent spectral overlap (2).

IRDye650LT can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos

labeled with IRDye650LT at the 5'-end can be used as PCR and Sanger DNA sequencing primers to generate
fluorescently-labeled PCR, sequencing or genetic analysis (AFLP, microsatellite) products (3-5).

Fluorophore Name
Absorbance Max, nm +/-10
Emission Max, nm +/-10

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Extinction Coefficient\*

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800



AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000



IRDye 75	0 NHS	756 7	76 260	0,000
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cy7.5 NHS 788 808 223,000

IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

- 1. Middendorf, L.R., Bruce, J.C., Eckles, R.D., Grone, D.L., Roemer, S.C., Sloniker, G.D., Steffens, D.L., Sutter, S.L., Brumbaugh, J.A., et al. Continuous, on-line DNA sequencing using a versatile infrared laser scanner/electrophoresis apparatus. *Electrophoresis* (1992), 13: 487-494.
- 2. Peng X., Chen, H., Draney, D.R., Volcheck, W., Schutz-Geschwender, A., Olive, D.M. A nonfluorescent, broad-range quencher dye for Forster resonance energy transfer assays. *Anal. Biochem.* (2009), 388: 220-228.
- 3. Yomano, L.P., Scopes, R.K., Ingram, L.O. Cloning, sequencing, and expression of the Zymomonas mobilis phosphoglycerate mutase gene (pgm) in Escherichia coli. *J. Bacteriol.* (1993), 175: 3926-3933.
- 4. Oetting, W.S., Lee, H.K., Flanders, D.J., Wiesner, T.A., King, R.A. Linkage Analysis with Multiplexed Short Tandem Repeat Polymorphisms Using Infrared Fluorescence and M13 Tailed Primers. *Genomics* (1995), 30: 450-458.

  5. Myburg, A.A., Remington, D.L, O'Malley, D.M., Sederoff, R.R., Whetton, R.W. High-Throughput AFLP Analysis Using Infrared Dye-Labeled Primers and an Automated DNA Sequencer. *Biotechniques* (2001), 30: 348-357.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### IRDye 680RD-N

Category Fluorescent Dyes

Modification Code IRD680RD-N

Reference Catalog Number 26-6647

Υ 5 Prime

3 Prime Υ

Internal Υ

fluorescent Hy per5 proprietary to GE Healthcare and its Molecular Weight(mw) 905.4 chemical structure currently is not

dve

ls

publicly available.

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. NHS Ligand conjugation requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Maleimide Ligand conjugation requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

IRDye700 is a near-IR fluorescent dye used for labeling oligonucleotides. IRDye800 has an absorbance maximum of 680 nm and an emission maximum of 694 nm. The combination of narrow absorbance/emission bands and low-background autofluorescence in the IR region results in higher S/N ratios and thus enhanced detection sensitivity compared with fluorophores with absorbance/emission maxima in the visible region (1). IRDye700 is used as a reporter moiety in real-time PCR applications. For such probes, IRDye700 is most commonly paired with the dark quencher QC-1, as the two have excellent spectral overlap (2).

IRDye700 can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos

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labeled with IRDye700 at the 5'-end can be used as PCR and Sanger DNA sequencing primers to generate
fluorescently-labeled PCR, sequencing or genetic analysis (AFLP, microsatellite) products (3-5).

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide
Fluorophore Name
Excitation Max, nm +/-10
Emission Max, nm +/-10
Extinction Coefficient*
Color**
Quencher
Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800



AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 699 715 120,000 Cy7 NHS 740 773 199,000 IRDye 750 NHS 756 776 260,000 cy7.5 NHS 788 808 223,000



#### IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

- 1. Middendorf, L.R., Bruce, J.C., Eckles, R.D., Grone, D.L., Roemer, S.C., Sloniker, G.D., Steffens, D.L., Sutter, S.L., Brumbaugh, J.A., et al. Continuous, on-line DNA sequencing using a versatile infrared laser scanner/electrophoresis apparatus. *Electrophoresis* (1992), 13: 487-494.
- 2. Peng X., Chen, H., Draney, D.R., Volcheck, W., Schutz-Geschwender, A., Olive, D.M. A nonfluorescent, broad-range quencher dye for Forster resonance energy transfer assays. *Anal. Biochem.* (2009), 388: 220-228.
- 3. Yomano, L.P., Scopes, R.K., Ingram, L.O. Cloning, sequencing, and expression of the Zymomonas mobilis phosphoglycerate mutase gene (pgm) in Escherichia coli. *J. Bacteriol.* (1993), 175: 3926-3933.
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Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### **IRDye 700**

Category Fluorescent Dyes

Modification Code IRD700

Reference Catalog Number 26-6672

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 769.9

CH<sub>3</sub> CH<sub>3</sub>

CH<sub>3</sub> H<sub>3</sub>C

N<sup>+</sup>

(CH<sub>2</sub>)<sub>6</sub>OH (CH<sub>2</sub>)<sub>4</sub>SO<sub>3</sub>

IRDye700 [26-6672-XX]

### Click here for a list of fluorophores.

IRDye700 is a near-IR fluorescent dye used for labeling oligonucleotides. IRDye800 has an absorbance maximum of 680 nm and an emission maximum of 694 nm. The combination of narrow absorbance/emission bands and low-background autofluorescence in the IR region results in higher S/N ratios and thus enhanced detection sensitivity compared with fluorophores with absorbance/emission maxima in the visible region (1). IRDye700 is used as a reporter moiety in real-time PCR applications. For such probes, IRDye700 is most commonly paired with the dark quencher QC-1, as the two have excellent spectral overlap (2).

IRDye700 can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with IRDye700 at the 5' end can be used as PCR and Sanger DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP, microsatellite) products (3-5). Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \*



genelink.com/newsite/products/rnaoligos.asp">Click here for RNA Oligos scale of synthesis and yield. NHS Ligand conjugation requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Maleimide Ligand conjugation requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne: Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal

sites. Click here for a list of click chemistry modifications. Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name Absorbance Max, nm +/-10 Emission Max, nm +/-10 Extinction Coefficient\* Color\*\* Quencher Cy5 650 665 250,000 IRDye 650 NHS 650 665 230,000 AZ647 NHS 655 680 191.800 AZ680 NHS 678 701 185,000



Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000 IRDye 750 NHS 756 776 260,000



IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

- 1. Middendorf, L.R., Bruce, J.C., Eckles, R.D., Grone, D.L., Roemer, S.C., Sloniker, G.D., Steffens, D.L., Sutter, S.L., Brumbaugh, J.A., et al. Continuous, on-line DNA sequencing using a versatile infrared laser scanner/electrophoresis apparatus. *Electrophoresis* (1992), 13: 487-494.
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# Oligo Modifications

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### IRDye 700-N

Category	Fluorescent Dyes	
Modification Code	IRD700-N	CH <sub>3</sub> CH <sub>3</sub>
Reference Catalog Number	26-6766	CH3 CH3 CH3
5 Prime	Υ	
3 Prime	Υ	N" V
Internal	Υ	$(CH_2)_6OH$ $(CH_2)_4SO_3^-$
Molecular Weight(mw)	754	IRDye700 [26-6672-XX]

IRDye 700 NHS is discontinued. See above related near IR fluorescent dyes.

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group.



Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

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Near Infrared	Fluorophore	Spectral	Data &	Quencher	Selection Gui	ide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient\*

Color\*\*

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800



AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000



IRDye 750	NHS	756 7	76 260	,000
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cy7.5 NHS 788 808 223,000

IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

- 1. Middendorf, L.R., Bruce, J.C., Eckles, R.D., Grone, D.L., Roemer, S.C., Sloniker, G.D., Steffens, D.L., Sutter, S.L., Brumbaugh, J.A., et al. Continuous, on-line DNA sequencing using a versatile infrared laser scanner/electrophoresis apparatus. *Electrophoresis* (1992), 13: 487-494.
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- 4. Oetting, W.S., Lee, H.K., Flanders, D.J., Wiesner, T.A., King, R.A. Linkage Analysis with Multiplexed Short Tandem Repeat Polymorphisms Using Infrared Fluorescence and M13 Tailed Primers. *Genomics* (1995), 30: 450-458.

  5. Myburg, A.A., Remington, D.L, O'Malley, D.M., Sederoff, R.R., Whetton, R.W. High-Throughput AFLP Analysis Using Infrared Dye-Labeled Primers and an Automated DNA Sequencer. *Biotechniques* (2001), 30: 348-357.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### IRDye 750-N

Category Fluorescent Dyes IRD750-N Modification Code CH<sub>3</sub> Reference Catalog Number 26-6648 CH<sub>3</sub> H<sub>3</sub>C 5 Prime 3 Prime Υ Internal Υ IRDye700 Molecular Weight(mw) 1094.2 [26-6672-XX]

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

IR750-NHS is a near-IR fluorescent dye used for labeling oligonucleotides. IR750 has an absorbance maximum of 756 nm and an emission maximum of 776 nm. The combination of narrow absorbance/emission bands and low-background autofluorescence in the IR region results in higher S/N ratios and thus enhanced detection sensitivity compared with fluorophores with absorbance/emission maxima in the visible region (1). IRDye750 is used as a reporter moiety in real-time PCR applications. For such probes, IRDye750 is most commonly paired with the dark quencher QC-1, as the two have excellent spectral overlap (2).

IRDye750 can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos

labeled with IRDye750 at the 5'-end can be used as PCR and Sanger DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP, microsatellite) products (3-5).	
Near Infrared Fluorophore Spectral Data & Quencher Selection Guide	

Fluorophore Name	
Absorbance Max, nm +/-10	
Emission Max, nm +/-10	

Color\*\*

Quencher

Cy5 650 665 250,000

Extinction Coefficient\*

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800



AZ680 NHS 678 701 185,000 Cy5.5 684 710 198,000 IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000



IRDye 750	NHS	756 7	76 260	,000
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cy7.5 NHS 788 808 223,000

IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

- 1. Middendorf, L.R., Bruce, J.C., Eckles, R.D., Grone, D.L., Roemer, S.C., Sloniker, G.D., Steffens, D.L., Sutter, S.L., Brumbaugh, J.A., et al. Continuous, on-line DNA sequencing using a versatile infrared laser scanner/electrophoresis apparatus. *Electrophoresis* (1992), 13: 487-494.
- 2. Peng X., Chen, H., Draney, D.R., Volcheck, W., Schutz-Geschwender, A., Olive, D.M. A nonfluorescent, broad-range quencher dye for Forster resonance energy transfer assays. *Anal. Biochem.* (2009), 388: 220-228.
- 3. Yomano, L.P., Scopes, R.K., Ingram, L.O. Cloning, sequencing, and expression of the Zymomonas mobilis phosphoglycerate mutase gene (pgm) in Escherichia coli. *J. Bacteriol.* (1993), 175: 3926-3933.
- 4. Oetting, W.S., Lee, H.K., Flanders, D.J., Wiesner, T.A., King, R.A. Linkage Analysis with Multiplexed Short Tandem Repeat Polymorphisms Using Infrared Fluorescence and M13 Tailed Primers. *Genomics* (1995), 30: 450-458.

  5. Myburg, A.A., Remington, D.L, O'Malley, D.M., Sederoff, R.R., Whetton, R.W. High-Throughput AFLP Analysis Using Infrared Dye-Labeled Primers and an Automated DNA Sequencer. *Biotechniques* (2001), 30: 348-357.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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### IRDye 800

Category	Fluorescent Dyes	IRDye800
Modification Code	IRD800	[26-6673-XX]
Reference Catalog Number	26-6673	CH3 CH3
5 Prime	Υ	-CH <sub>3</sub> H <sub>3</sub> C
3 Prime	N	N+
Internal	N	(ch <sub>2</sub> ) <sub>6</sub> он о (ch <sub>2</sub> ) <sub>4</sub> so <sub>3</sub>
Molecular Weight(mw)	878.1	

### Click here for a list of fluorophores.

IRDye series of dyes are near-IR fluorescent dye used for labeling oligonucleotides. IRDye800 has an absorbance maximum of 795 nm and an emission maximum of 812 nm. The combination of narrow absorbance/emission bands and low-background autofluorescence in the IR region results in higher S/N ratios and thus enhanced detection sensitivity compared with fluorophores with absorbance/emission maxima in the visible region (1).

IRDye800 can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with IRDye800 at the 5'-end can be used as PCR and Sanger DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP, microsatellite) products (3-5). Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group.



Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Maleimide Ligand conjugation requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO: Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide Fluorophore Name Absorbance Max, nm +/-10 Emission Max, nm +/-10 Extinction Coefficient\* Color\*\* Quencher Cy5 650 665 250,000 IRDye 650 NHS 650 665 230,000 AZ647 NHS 655 680 191,800 AZ680 NHS 678 701 185,000



Cy5.5 684 710 198,000

IRDye 700 NHS 684 710 288,000 AZdye700 NHS 696 719 192,000 Atto 700 NHS 700 716 120,000 Atto 725 NHS 728 751 120,000 Atto 740 NHS 743 763 120,000 Cy7 NHS 740 773 199,000 IRDye 750 NHS 756 776 260,000



IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

- 1. Middendorf, L.R., Bruce, J.C., Eckles, R.D., Grone, D.L., Roemer, S.C., Sloniker, G.D., Steffens, D.L., Sutter, S.L., Brumbaugh, J.A., et al. Continuous, on-line DNA sequencing using a versatile infrared laser scanner/electrophoresis apparatus. *Electrophoresis* (1992), 13: 487-494.
- 2. Peng X., Chen, H., Draney, D.R., Volcheck, W., Schutz-Geschwender, A., Olive, D.M. A nonfluorescent, broad-range quencher dye for Forster resonance energy transfer assays. *Anal. Biochem.* (2009), 388: 220-228.
- 3. Yomano, L.P., Scopes, R.K., Ingram, L.O. Cloning, sequencing, and expression of the Zymomonas mobilis phosphoglycerate mutase gene (pgm) in Escherichia coli. *J. Bacteriol.* (1993), 175: 3926-3933.
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# Oligo Modifications

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### IRDye 800RS-N

Category	Fluorescent Dyes	IRDye800
Modification Code	IRD800RS-N	[26-6673-XX]
Reference Catalog Number	26-6767	CH3 CH3
5 Prime	Υ	-CH <sub>3</sub> H <sub>3</sub> C
3 Prime	Υ	N+
Internal	Υ	(ch <sub>2</sub> ) <sub>6</sub> он о (ch <sub>2</sub> ) <sub>4</sub> so <sub>3</sub>
Molecular Weight(mw)	864	

### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.



Click here for a list of conjugation chemistry modifications. Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

IRDye series of dyes are near-IR fluorescent dye used for labeling oligonucleotides. IRDye800 has an absorbance maximum of 767 nm and an emission maximum of 786 nm. The combination of narrow absorbance/emission bands and low-background autofluorescence in the IR region results in higher S/N ratios and thus enhanced detection sensitivity compared with fluorophores with absorbance/emission maxima in the visible region (1).

IRDye800 can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos

labeled with IRDye800 at the 5'-end can be used as PCR and Sanger DNA sequencing primers to generate
fluorescently-labeled PCR, sequencing or genetic analysis (AFLP, microsatellite) products (3-5).

Fluorophore Name	
Absorbance Max, nm +/-10	
Emission Max, nm +/-10	

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Color\*\*

Quencher

Cy5 650 665 250,000

Extinction Coefficient\*

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800



AZ680 NHS 678 701 185,000	
Cy5.5 684 710 198,000	
IRDye 700 NHS 684 710 288,000	
AZdye700 NHS 696 719 192,000	
Atto 700 NHS 700 716 120,000	
Atto 725 NHS 728 751 120,000	
Atto 740 NHS 743 763 120,000	
Cy7 NHS 740 773 199,000	



IRDye 750 NHS 756 776 20
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cy7.5 NHS 788 808 223,000

IRDye 800 NHS 795 819 240,000

 $^*$  Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

- 1. Middendorf, L.R., Bruce, J.C., Eckles, R.D., Grone, D.L., Roemer, S.C., Sloniker, G.D., Steffens, D.L., Sutter, S.L., Brumbaugh, J.A., et al. Continuous, on-line DNA sequencing using a versatile infrared laser scanner/electrophoresis apparatus. *Electrophoresis* (1992), 13: 487-494.
- 2. Peng X., Chen, H., Draney, D.R., Volcheck, W., Schutz-Geschwender, A., Olive, D.M. A nonfluorescent, broad-range quencher dye for Forster resonance energy transfer assays. *Anal. Biochem.* (2009), 388: 220-228.
- 3. Yomano, L.P., Scopes, R.K., Ingram, L.O. Cloning, sequencing, and expression of the Zymomonas mobilis phosphoglycerate mutase gene (pgm) in Escherichia coli. *J. Bacteriol.* (1993), 175: 3926-3933.
- 4. Oetting, W.S., Lee, H.K., Flanders, D.J., Wiesner, T.A., King, R.A. Linkage Analysis with Multiplexed Short Tandem Repeat Polymorphisms Using Infrared Fluorescence and M13 Tailed Primers. *Genomics* (1995), 30: 450-458.

  5. Myburg, A.A., Remington, D.L., O'Malley, D.M., Sederoff, R.R., Whetton, R.W. High-Throughput AFLP Analysis
- 5. Myburg, A.A., Remington, D.L., O'Malley, D.M., Sederoπ, R.R., Whetton, R.W. High-Throughput AFLP Analys Using Infrared Dye-Labeled Primers and an Automated DNA Sequencer. *Biotechniques* (2001), 30: 348-357.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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### Joe

4,5-dichloro-dimethoxy-fluorescein (JOE) is a dichlorinated, dimethoxylated version of the fluorescent dye fluorescein, and is used for labeling oligonucleotides at either the 5'- or 3'-end, or internally (via an NHS ester). JOE has an absorbance maximum of 520 nm and an emission maximum of 548 nm. JOE can be used in real-time PCR applications as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, JOE is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap. JOE-labeled primers have also been used for bacterial SNP genotyping by allele-specific real-time PCR (4).

JOE also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with JOE at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. NOTE: If JOE is on the 3'-end of the oligo, it cannot be used as a primer in PCR-based applications.

Note that for internal labeling of an oligo, the NHS ester form of JOE must be used. Consequently, the oligo first must be synthesized with the Amino C6 version of the base phosphoramidite (for example, Amino-C6-dA, Amino-C6-dC, etc.) at the desired position to be labeled with JOE. The appropriate JOE-NHS ester is then manually attached to the oligo through that base's C6 amino group in a separate reaction post-synthesis. **References** 

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995) **4**: 1-6
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308. 4. Huygens, F., Inman-Bamber, J., Nimmo-G.R., Munckhof, W., Schooneveldt, J., Harrison, B., McMahon, J.A., Giffard, P.M. Staphylococcus aureus Genotyping Using Novel Real-Time PCR Formats. *J. Clin. Microbiol.*



(2006), 44: 3712-3718.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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#### LC Cyan 500-N

Category Fluorescent Dyes

Modification Code LC-C500-N

Reference Catalog Number 26-6688

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 580

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Cyan 500 NHS ester is an amine-reactive fluorescent label. It is designed and recommended by Roche for application in LightCycler® instruments. The dye is used to label amino-modified oligonucleotides which can be detected on excitation at 430 nm in Roche LightCycler® instruments.

Fluorescence properties of LC Cyan 500 has an Excitation max of 436 nm and Emission max at 481 nm. A close substitution will be AF488 with Excitation max of 494 nm and Emission max at 517 nm or Fam Excitation max of 495 nm and Emission max at 520 nm.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### LC Red 610 N

Category Fluorescent Dyes

Modification Code LC610-N

Reference Catalog Number 26-6675

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 0



#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



LC Red 610 NHS Ester is a fluorescent dye for labeling oligonucleotide hybridization probes used in LightCycler qPCR assays. LC Red 610 has an absorbance maximum of 590 nm and an emission maximum of 610 nm. LightCycler ("adjacent probe") assays require two single-stranded hybridization probes which bind to adjacent sites on a target strand. One probe is 3'-end-labeled with a donor fluorophore (typically fluorescein), while the other probe is 5'-end labeled with LC Red 610 as the acceptor fluorophore (and blocked at its 3'-end with phosphate). The distance between the 3' and 5'-ends of the respective probes, when hybridized to the target, is carefully chosen to ensure efficient fluorescence resonance energy transfer (FRET) between donor and acceptor fluorophores. When not hybridized to the target, that is, when they are "free-floating" in solution, no FRET should occur, and only the fluorescence of the donor fluorophore should be present. However, when both probes are hybridized to the target, FRET should occur, resulting in a decrease in donor fluorescence and increase in acceptor fluorescence (1).

LightCycler assay systems have been developed for SNP detection (2), allelic discrimination (3), gene copy determination (4), pathogen detection (5), viral load quantification (6), and gene expression analysis (7). Additional red dyes suitable for use as acceptor fluorophores, namely LC Red 640 and Cy5.5, permit LightCycler assays to be in multiplex format. **References** 1. Wittwer, C.T., Herrmann, M.G., Moss, A.A., Rasmussen, R.P. Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques* (1997), **22**: 130-131.

- 2. Hiratsuka, M., Narahara, K., Kishikawa, Y., Ismail, H.S., Endo, N., Agatsuma, Y., Matsuura, M., Inoue, T., Tomioka, Y., Mizugaki, M. A simultaneous LightCycler detection assay for five genetic polymorphisms influencing drug sensitivity. *Clin. Biochem.* (2002), **35**:35-40.
- 3. Mangasser-Stephan, K., Tag, C., Reiser, A., Gressner, A.M. Rapid Genotyping of Hemochromatosis Gene Mutations on the LightCycler with Fluorescent Hybridization Probes. *Clin. Chem.* (1999), **45**: 1875-1878.
- 4. Ruiz-Ponte, C., Loidi, L., Vega, A., Carracedo, A., Barros, F. Rapid Real-Time Fluorescent PCR Gene Dosage Test for the Diagnosis of DNA Duplications and Deletions. *Clin. Chem.* (2000), **46**: 1574-1582.
- 5. Wellinghausen, N., Wirths, B., Franz, A.R., Karolyi, L., Marre, R., Reischl, U. Algorithm for the identification of bacterial pathogens in positive blood cultures by real-time LightCycler polymerase chain reaction (PCR) with sequence-specific probes. *Diagn. Microbiol. Infect. Dis.* (2004), **48**: 229-241.
- 6. Gulley, M.L., Fan, H., Elmore, S.H. Validation of Roche LightCycler Epstein-Barr virus quantification reagents in a clinical laboratory setting. *J. Mol. Diagn.* (2006), **8**: 589-597.
- 7. Frade, J.P., Warnock, D.W., Arthington-Skaggs, B.A. Rapid Quantification of Drug Resistance Gene Expression in Candida albicans by Reverse Transcriptase LightCycler PCR and Fluorescent Probe Hybridization. *J. Clin. Microbiol.* (2004), **42**: 2085-2093.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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#### LC Red 640 N

Category Fluorescent Dyes

Modification Code LC640-N

Reference Catalog Number 26-6677

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 0



#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



LC Red 640 NHS Ester is a fluorescent dye for labeling oligonucleotide hybridization probes used in LightCycler qPCR assays. LC Red 640 has an absorbance maximum of 625 nm and an emission maximum of 640 nm. LightCycler ("adjacent probe") assays require two single-stranded hybridization probes which bind to adjacent sites on a target strand. One probe is 3'-end-labeled with a donor fluorophore (typically fluorescein), while the other probe is 5'-end labeled with LC Red 640 as the acceptor fluorophore (and blocked at its 3'-end with phosphate). The distance between the 3' and 5'-ends of the respective probes, when hybridized to the target, is carefully chosen to ensure efficient fluorescence resonance energy transfer (FRET) between donor and acceptor fluorophores. When not hybridized to the target, that is, when they are "free-floating" in solution, no FRET should occur, and only the fluorescence of the donor fluorophore should be present. However, when both probes are hybridized to the target, FRET should occur, resulting in a decrease in donor fluorescence and increase in acceptor fluorescence (1).

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- 3. Mangasser-Stephan, K., Tag, C., Reiser, A., Gressner, A.M. Rapid Genotyping of Hemochromatosis Gene Mutations on the LightCycler with Fluorescent Hybridization Probes. *Clin. Chem.* (1999), **45**: 1875-1878.
- 4. Ruiz-Ponte, C., Loidi, L., Vega, A., Carracedo, A., Barros, F. Rapid Real-Time Fluorescent PCR Gene Dosage Test for the Diagnosis of DNA Duplications and Deletions. *Clin. Chem.* (2000), **46**: 1574-1582.
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- 7. Frade, J.P., Warnock, D.W., Arthington-Skaggs, B.A. Rapid Quantification of Drug Resistance Gene Expression in Candida albicans by Reverse Transcriptase LightCycler PCR and Fluorescent Probe Hybridization. *J. Clin. Microbiol.* (2004), 42: 2085-2093.





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# Oligo Modifications

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#### MarBlue-460 NHS

Category Fluorescent Dyes

Modification Code MarBI-460-N

Reference Catalog Number 26-6687

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 284.22

MBlue-460NHS [26-6687]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



MBlue-460 NHS is 6,8-Difluoro-7-hydroxy-4-methylcoumarin NHS Ester exhibiting bright blue fluorescence emission near 460 nm is optimally excited by the intense 365 nm spectra line of the mercury-arc lamp and detect optimally with DAPI optical filter sets. Because the pKa value of 6,8-Difluoro-7-hydroxy-4-methylcoumarin derivatives are 2-3 log units lower compared to those of the corresponding 7-hydroxycoumarin conjugates, 6,8-Difluoro-7-hydroxy-4-methylcoumarin conjugates are strongly fluorescent even at neutral pH.

MBlue-460 is an NHS and is available as a fluorescent dye that can be conjugated to an oligo, either RNA or DNA. This is a post synthesis conjugation to a primary amino group. The amino group can be placed at the 5' and 3' and for internal positions an amino modified base is used, e.g Amino dT C6

MBlue-460 can be used to create blue-fluorescent bioconjugates. Based on the 6,8-difluoro-7-hydroxycoumarin fluorophore, dye exhibits bright blue fluorescence emission near 460 nm and is optimally excited by the intense 365 nm spectra line of the mercury-arc lamp.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Methylene Blue Azide

Category Redox Electrochemical Modification Code MB-N3 CF<sub>3</sub>COO Reference Catalog Number 26-6988 5 Prime 3 Prime Υ Azide click to DBCO Internal Υ BCN or Alkyne Molecular Weight(mw) 553 Methylene Blue Azide Oligo [26-6988-XX]

This modification is a post synthesis conjugation to BCN, alkyne or DBCO modification at the appropriate site for click conjugation. Gene Link offers post synthesis click free conjugation to oligos labelled with BCN at the 5' or 3' end. The azide group of Methylene Blue is linked to BCN group on the oligo. BCN group is required on the oligo. Additional charges applies for BCN

#### BCN-3 BCN-5

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites.

Click here for a list of conjugation chemistry modifications. Maleimide Ligand conjugation requires a thiol group.



Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.

Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide,

BCN:Tetrazine and TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Methylene Blue Azide is a derivative of the well-known redox dye Methylene Blue. The azide derivative enables use in copper free click chemistry reactions with DBCO labelled reactants.

The dye can be reversibly reduced to the colorless leuko form. Upon oxidation (e.g. with oxygen) the dye recovers, and the absorption is fully restored.

Methylene Blue (e.g Atto MB2) NHS is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5' or 3'-end of an oligonucleotide, enables the modified oligo's use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For "signal-on" sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For "signal-off" sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.

Methylene blue is a pH indicator that changes color depending on the acidity or alkalinity of a solution. In acidic conditions (pH < 6), it appears blue, while in neutral to basic conditions (pH > 7), it can shift to a colorless or light blue form. This transition is due to changes in the molecular structure of methylene blue, which affects its light absorption properties.

#### References

- 1. Ricci, F., Lai, R.Y., Plaxco, K.W. Linear, redox modified DNA probes as electrochemical DNA sensors. *Chem. Comm.* (2007), **36**: 3768-3770.
- 2. Song, S., Wang, L., Li, J., Zhao, J., Fan, C. Aptamer-based biosensors. Trends in Anal. Chem. (2008), 27: 108-117.
- 3. Ferapontova, E.E., Gothelf, K.V. Optimization of the Electrochemical RNA-Aptamer Based Biosensor for Theophylline by Using a Methylene Blue Redox Label. *Electroanalysis* (2009), **21**: 1261-1266.
- 4. Xiao, Y., Lubin, A.A., Heeger, A.J., Plaxco, K.W.. Label-free Electronic Detection of Thrombin in Blood Serum by Using an Aptamer-Based Sensor. *Angew. Chem. Int. Ed. Engl.* (2005), **44**: 5456-5459..

#### Copper-free Click Chemistry Modifications

Use azide modified oligos with DBCO Cyclooctyne-based modifications for ease of copper-free click reagents. These are simple to use and has excellent click performance in 17 hours or less at room temperature. Gene Link offers 5'-DBCO-TEG for preparing oligos with 5'-DBCO and a 15 tom triethylene glycol spacer arm, DBCO-dT for inserting a DBCO group at any position within the oligonucleotide and DBCO-sulfo-NHS Ester is also offered for post-synthesis conjugation reactions. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqeous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.



Azide C3 is available to introduce a stable azide group at the 3' of an oligo. Use Azide butyrate NHS [26-6922] for introduction of azide at internal or 5' position by conjugating to an amino-modified oligonucleotide. Introduction can be done at either the 5'- or 3'-end, or internally. To do this, the oligo first must be synthesized with a primary amino functional group modification, e.g Amino C6 for the 5' end or amino C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azidobutyrate NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The presence of the azide allows the user to use "Click Chemistry" (a [3+2] cycloaddition reaction between alkynes and azides, using copper (I) iodide as a catalyst) to conjugate the azide-modified oligo to a terminal alkyne-modified oligo with extremely high regioselectivity and efficiency (1,2). Preparation of the alkyne-modified oligo can be achieved using the 5'-Hexynyl modifier (see its respective tech sheet for details). Click chemistry can be used to form short, cyclic oligos that can be used as research tools in various biophysical and biological studies (3). In particular, they have considerable potential for in vivo work, as cyclic oligos are known to be very stable in serum for up to several days.

#### References

- 1. Huisgen, R. Angew. Chem. Int. Ed. (1963), 2]: 565-568.
- 2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599. 3. Kumar, R., El-Sagheer, A., Tumpane, J., Lincoln, P., Wilhelmsson, L.M., Brown, T. Template-Directed Oligonucleotide Strand Ligation, Covalent Intramolecular DNA Circularization and Catenation Using Click Chemistry. *J. Am. Chem. Soc.* (2007), **129**: 6859-6864.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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#### **Methylene Blue II**

Category Redox Electrochemical

Modification Code MB-II

Reference Catalog Number 26-6909

5 Prime Y

3 Prime N

Internal N

\$250 charge per order.

Molecular Weight(mw) 489.57

26-6909-XX

Some branching off the Methylene Blue II will occur when incorporated internally within an oligo, we recommend Methylene Blue II be placed at or near the 5' terminus of the oligo sequence. Methylene blue II modification is an amidite that can be directly coupled by using automated chemistry. This requires the use of mild reagents for synthesis and there is an additional charge of

Methylene Blue (MB) NHS is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5' or 3'-end of an oligonucleotide, enables the modified oligo's use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For "signal-on" sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For "signal-off" sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.

Methylene blue is a pH indicator that changes color depending on the acidity or alkalinity of a solution. In acidic conditions (pH < 6), it appears blue, while in neutral to basic conditions (pH > 7), it can shift to a colorless or light blue form. This transition is due to changes in the molecular structure of methylene blue, which affects its light absorption properties.

#### References

1. Ricci, F., Lai, R.Y., Plaxco, K.W. Linear, redox modified DNA probes as electrochemical DNA sensors.



Chem. Comm. (2007), 36: 3768-3770.

- 2. Song, S., Wang, L., Li, J., Zhao, J., Fan, C. Aptamer-based biosensors. Trends in Anal. Chem. (2008), 27: 108-117.
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### Oligo Modifications

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#### Methylene Blue Maleimide

Category Redox Electrochemical

Modification Code MB2-Mal

Reference Catalog Number 26-6526

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 478

Methylene blue maleimide modification is a post synthesis conjugation to thiol group. The thiol group can be placed at the 5' and 3' and for internal positions a thiol dT C6 is used.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5'. 3' and internal sites.

Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.

Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide,

BCN:Tetrazine and TCO:Tetrazine.



Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Methylene Blue (MB) is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5' or 3'-end of an oligonucleotide, enables the modified oligo's use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For "signal-on" sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For "signal-off" sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.

Methylene blue is a pH indicator that changes color depending on the acidity or alkalinity of a solution. In acidic conditions (pH < 6), it appears blue, while in neutral to basic conditions (pH > 7), it can shift to a colorless or light blue form. This transition is due to changes in the molecular structure of methylene blue, which affects its light absorption properties.

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### Oligo Modifications

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#### Methylene blue MB2-NHS

Category	Redox Electrochen	nical
Modification Code	MB2-N	N.
Reference Catalog Number	26-6908	H <sub>3</sub> C CH <sub>3</sub>
5 Prime	Υ	5' Amino Linker C6
3 Prime	Υ	H <sub>3</sub> C 3 Amino Linker C6 [26-6418-XX]
Internal	Υ	O NH P-vvvOligo-3'
Molecular Weight(mw)	338.4	5' Methylene Blue NHS Ester [26-6908-XX]

Click here for a list of other Redox Electrochemical Modifications.

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation\* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol \* The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. \* Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications.



**Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites. Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN: TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

Methylene Blue (e.g Atto MB2) NHS is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5' or 3'-end of an oligonucleotide, enables the modified oligo's use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For "signal-on" sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For "signal-off" sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.

Methylene blue is a pH indicator that changes color depending on the acidity or alkalinity of a solution. In acidic conditions (pH < 6), it appears blue, while in neutral to basic conditions (pH > 7), it can shift to a colorless or light blue form. This transition is due to changes in the molecular structure of methylene blue, which affects its light absorption properties.

#### References

- 1. Ricci, F., Lai, R.Y., Plaxco, K.W. Linear, redox modified DNA probes as electrochemical DNA sensors. *Chem. Comm.* (2007). **36**: 3768-3770.
- 2. Song, S., Wang, L., Li, J., Zhao, J., Fan, C. Aptamer-based biosensors. *Trends in Anal. Chem.* (2008), 27: 108-117.
- 3. Ferapontova, E.E., Gothelf, K.V. Optimization of the Electrochemical RNA-Aptamer Based Biosensor for Theophylline by Using a Methylene Blue Redox Label. *Electroanalysis* (2009), **21**: 1261-1266.
- 4. Xiao, Y., Lubin, A.A., Heeger, A.J., Plaxco, K.W.. Label-free Electronic Detection of Thrombin in Blood Serum by Using an Aptamer-Based Sensor. *Angew. Chem. Int. Ed. Engl.* (2005), **44**: 5456-5459..





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# Oligo Modifications

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#### PBlue-455 NHS

Category Fluorescent Dyes

Modification Code PBlue-455

Reference Catalog Number 26-6524

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 339.21

NH-Oligo-3'

PBlue-455 NHS Oligo [26-6524-XX]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



PBlue-455 is a UV-excitable, bright blue fluorescent dye used for labeling oligonucleotides excitable by the 407 nm spectral line of blue diode (violet color) laser. PBlue-455 has an absorbance maximum of 410 nm and an emission maximum of 455 nm. Because UV light can photo damage labeled oligos, and many kinds of cells and tissues autofluoresce under UV light, PBlue-455 can only be used in a limited number of applications. Nevertheless, for such applications as nucleic acid microarrays and in situ hybridization, where a blue fluorescent probe provides a easily distinguishable, contrasting color to the green, yellow, orange and red fluorescence produced by longer-wavelength probes, PBlue-455 can be a good choice.





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### Oligo Modifications

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#### Quasar 570-3'

СНз H<sub>3</sub>C CH<sub>3</sub> H<sub>3</sub>C Category Fluorescent Dyes Modification Code Qsr-570 Reference Catalog Number 26-6567 5 Prime Ν 3 Prime Υ Internal Ν Oligo-5' .....-Quasar 570 3' /-Cy3 Molecular Weight(mw) 620.74 [26-6567-XX] OH

Quasar 570 is identical to Cy3 and Quasar 670 is identical to Cy5. Cy3 and Cy5 named fluorophores are available for 5', 3', Internal and NHS forms for post synthesis conjugation. Please see related fluorophores listed above. Click here for list of fluorophores.

Quasar 570 is an indocarbocyanine which fluoresces in the yellow-orange region of the visible spectrum. This fluorophore is a direct replacement for Cy3 dye. Quasar 570 amidite is used for the 5' labeling of fluorogenic probes used in 5' nuclease assays, Molecular Beacons, Scorpions primers, and other detection assays. BHQ-2 dye will quench the Quasar 570 moiety. For internal labeling we recommend the use of Cy3-NHS that is conjugated post synthesis using an amino group. BR>

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers \*\*Black Hole Quencher License Agreement "Black Hole Quencher", "BHQ-1", "BHQ-2" and "BHQ-3" are registered trademarks of Biosearch Technologies, Inc., Novato, CA. The BHQ technology is licensed and sold under agreement with Biosearch and these products are sold exclusively for R&D use by the purchaser. They may not be used for clinical or diagnostic purposes and they may not be resold, distributed or re-packaged.





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### Oligo Modifications

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#### **Quasar 670-3'**

Category Fluorescent Dyes Modification Code Qsr-670 Reference Catalog Number 26-6709 5 Prime Ν 3 Prime Υ Internal Ν Quasar-670-3' /Cy5 Oligo-5' www-Molecular Weight(mw) 645.78 [26-6709-XX] OH

Quasar 570 is identical to Cy3 and Quasar 670 is identical to Cy5. Cy3 and Cy5 named fluorophores are available for 5', 3', Internal and NHS forms for post synthesis conjugation. Please see related fluorophores listed above. Click here for list of fluorophores.

Quasar 670 is an indocarbocyanine which fluoresces in the red region of the visible spectrum. The absorption Max is 644 nm and emission max is at 670 nm. This fluorophore is a direct replacement for Cy5. Quasar 670 can be used for the 5' labeling of fluorogenic probes used in 5' nuclease assays, Molecular Beacons, and other detection assays. Appropriate quencher for Quaser 670 is BBQ 650 with an absorption max at 650nm, BHQ-2 has an absorption range 550-650 nm with the max at 579 nm; it will also quench the fluorescence of Quasar 670.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730 BBQ-650 650 550-750 Click here for complete list of quenchers \*\*Black Hole Quencher License Agreement "Black Hole Quencher", "BHQ-1", "BHQ-2" and "BHQ-3" are registered trademarks of Biosearch Technologies, Inc., Novato, CA. The BHQ technology is licensed and sold under agreement with Biosearch and these products are sold exclusively for R&D use by the purchaser. They may not be used for clinical or diagnostic purposes and they may not be resold, distributed or re-packaged.





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### Oligo Modifications

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#### **ROX N**

Category Fluorescent Dyes

Modification Code ROX-N

Reference Catalog Number 26-6430

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 516.6

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



6-Carboxyl-X-Rhodamine (ROX) is a red fluorescent dye used for labeling oligonucleotides. ROX has an absorbance maximum of 588 nm and an emission maximum of 608 nm. ROX plays an important role in real-time PCR applications, being primarily used as the passive reference dye for normalization of the fluorescent signals produced by the dye(s) attached to the various probes (TaqMan (1), Molecular Beacon (2), etc.) as reporter dyes. Such normalization is needed to correct for well-to-well signal fluctuations (which are often due to the design of the instrument). If another dye is used as the reference, a ROX-modified oligo probe can then be used in real-time PCR assays. In such cases, ROX is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

ROX can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with ROX at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

Because ROX currently only is produced in the form of an NHS ester, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The ROX NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis.

Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

Emission max (nm) VIC Pink Red 538 554 Cal Orange 560 Pink Red 537 558 HEX Pink Red 535 556 NED Red Orange 546 575 Cy3 Red Orange 550 570 PET Red Orange 558 595 Cy3.5 Red 588 604 ROX Red 575 602 CAL Fluor Red 590 Red 569 591 Texas Red Red 583 603

# Click here for a list of fluorophores. References

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





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### Oligo Modifications

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#### Tamra N

Category	Fluorescent Dyes	
Modification Code	Tamra-N	$\stackrel{\downarrow}{N}$ , $\stackrel{\downarrow}{\wedge}$ $\stackrel{\downarrow}{\circ}$
Reference Catalog Number	26-6450	(III)
5 Prime	Υ	0.
3 Prime	Υ	
Internal	Υ	
Molecular Weight(mw)	527.53	Tamra NHS [26-6450-XX]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Carboxytetramethylrhodamine (TAMRA) is a fluorescent dye that is a derivative of rhodamine, and is used to label oligonucleotides at the 5'- or 3'-ends, or internally. TAMRA has an absorbance maximum of 565 nm and an emission maximum of 580 nm. TAMRA-modified oligonucleotides play a particularly important role in both fluorescence resonance energy transfer (FRET) and real-time PCR applications.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

[26-6451-XX]

# Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

#### Tamra-3'

Category Fluorescent Dyes

Modification Code 3-Tamra

Reference Catalog Number 26-6451

5 Prime I

3 Prime Y

Internal I

Molecular Weight(mw) 527.53

TAMRA (Carboxytetramethy Irhodamine)

Carboxytetramethylrhodamine (TAMRA) is a fluorescent dye that is a derivative of rhodamine, and is used to label oligonucleotides at the 5' or 3' ends, or internally. TAMRA has an absorbance maximum of 565 nm and an emission maximum of 580 nm. TAMRA-modified oligonucleotides play a particularly important role in both fluorescence resonance energy transfer (FRET) and real-time PCR applications.

FRET is a distance-dependent interaction between two dye molecules in which excitation is radiationlessly transferred from one dye (the donor) to the second dye (the acceptor), due to spectral overlap. Because the efficiency of the energy transfer is extremely sensitive to the distance between the molecules (varying as the inverse sixth power of that distance) (1), FRET can be used to study biological phenomena that produce changes in molecular proximity (2). For oligonucleotides slated for use in FRET application, a common donor-acceptor pair is 6-FAM (donor) / TAMRA (acceptor), due to their good spectral overlap. As the donor, 6-FAM is excited at 492 nm and transfers this energy to TAMRA, which then emits light at 580 nm. FRET oligo probes are widely used to monitor biochemical reactions, particularly in in vivo studies (3).

Besides being used as a FRET fluorophore, TAMRA also can be used as a FRET-based quencher moiety in real-time PCR probes such as TaqMan probes (4), Scorpion primers (5) and Molecular Beacons (6). For such probes, 6-FAM is used as the reporter moiety, and its emission at 521 nm is monitored. When 6-FAM and TAMRA are in close proximity, the former's fluorescence at 521 nm is quenched by the latter. After sufficient spatial separation of the two dyes during the course of the assay, 6-FAM's fluorescence is no longer quenched, and its fluorescence signal becomes observable.

TAMRA also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with TAMRA at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

Note that, because TAMRA is in the form of an NHS ester, the oligo first must be synthesized with an Amino C6 Linker (for the ends) or the Amino C6 version of the base phosphoramidite (for internal labeling). The TAMRA-NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis.



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#### References

- 1. Stryer, L., Haugland, R.P. Energy transfer: a spectroscopic ruler. Proc. Natl. Acad. Sci. USA (1967), 58: 719-726.
- 2. Wu, P., Brand, L. Resonance energy transfer: methods and applications. Anal. Biochem. (1994), 218: 1-13.
- 3. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
- 4. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 5. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 6. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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#### Tamra-5'

Category Fluorescent Dyes

Modification Code 5-Tamra

Reference Catalog Number 26-6947

5 Prime Y
3 Prime N

Internal N

Molecular Weight(mw) 527.53

P)vvvOligo-3

5'-TAMRA (Carboxytetramethy Irhodamine) [26-6947-XX]

Carboxytetramethylrhodamine (TAMRA) is a fluorescent dye that is a derivative of rhodamine, and is used to label oligonucleotides at the 5'- or 3'- ends, or internally. TAMRA has an absorbance maximum of 565 nm and an emission maximum of 580 nm. TAMRA-modified oligonucleotides play a particularly important role in both fluorescence resonance energy transfer (FRET) and real-time PCR applications.

FRET is a distance-dependent interaction between two dye molecules in which excitation is radiationlessly transferred from one dye (the donor) to the second dye (the acceptor), due to spectral overlap. Because the efficiency of the energy transfer is extremely sensitive to the distance between the molecules (varying as the inverse sixth power of that distance) (1), FRET can be used to study biological phenomena that produce changes in molecular proximity (2). For oligonucleotides slated for use in FRET application, a common donor-acceptor pair is 6-FAM (donor) / TAMRA (acceptor), due to their good spectral overlap. As the donor, 6-FAM is excited at 492 nm and transfers this energy to TAMRA, which then emits light at 580 nm. FRET oligo probes are widely used to monitor biochemical reactions, particularly in in vivo studies (3).

Besides being used as a FRET fluorophore, TAMRA also can be used as a FRET-based quencher moiety in real-time PCR probes such as TaqMan probes (4), Scorpion primers (5) and Molecular Beacons (6). For such probes, 6-FAM is used as the reporter moiety, and its emission at 521 nm is monitored. When 6-FAM and TAMRA are in close proximity, the former's fluorescence at 521 nm is quenched by the latter. After sufficient spatial separation of the two dyes during the course of the assay, 6-FAM's fluorescence is no longer quenched, and its fluorescence signal becomes observable.

TAMRA also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with TAMRA at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

Note that, because TAMRA is in the form of an NHS ester, the oligo first must be synthesized with an Amino C6 Linker (for the ends) or the Amino C6 version of the base phosphoramidite (for internal labeling).



The TAMRA-NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. **References** 

- 1. Stryer, L., Haugland, R.P. Energy transfer: a spectroscopic ruler. Proc. Natl. Acad. Sci. USA (1967), 58: 719-726.
- 2. Wu, P., Brand, L. Resonance energy transfer: methods and applications. Anal. Biochem. (1994), 218: 1-13.
- 3. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
- 4. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 5. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 6. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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#### Tamra-dT

Carboxytetramethylrhodamine-deoxythymidien (TAMRA-dT) is a deoxythymidine nucleoside derivitized with TAMRA through a spacer arm. TAMRA-dT is used to internally label an oligonucleotide at a dT position. TAMRA-dT has an absorbance maximum of 565 nm and an emission maximum of 580 nm. TAMRA-dT can be used to internally label a Fluorescence Resonance Energy Transfer (FRET) DNA oligonucleotide probe with a quencher moiety. Such a labeling strategy is pertinent in cases where the distance between the quencher and fluorophore needs optimization for efficient quenching. For such probes, 6-FAM is most commonly used as the reporter moiety as the two dyes have excellent spectral overlap.

TAMRA-dT also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos internally labeled with TAMRA-dT also can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. For further details concerning the TAMRA dye, please see the technical sheet for it.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

OH

# Oligo Modifications

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#### Tet-5'

Category	Fluorescent Dyes	
Modification Code	Tet-5	a o a
Reference Catalog Number	26-6433	CI
5 Prime	Υ	
3 Prime	N	HN A
Internal	N	Pwww Oligo 3'
Molecular Weight(mw)	675.24	5'-Tetrachloro-Fluorescein (TET) [26-6433-XX]

#### Click here for a list of fluorophores.

Tetrachloro fluorescein (TET) is tetra-chloro derivative of fluorescein that is used to fluorescently label oligonucleotides. TET has an absorbance maximum of 522 nm and an emission maximum of 538 nm. TET plays a role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, TET is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap.

TET can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with TET at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References** 

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995) 4: 1-6
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyaqi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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#### **Tet-Azide**

Category Click Chemistry

Modification Code Tet-N3

Reference Catalog Number 26-6724

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 596.2

> 6-TET A zide [26-6724-XX]

This modification is a post synthesis conjugation to an alkyne or DBCO modification at the appropriate site for click conjugation.

TET (Tetrachloro fluorescein)-Azide is a fluorescent dye containing a terminal azide group. TET has an absorbance maximum of 522 nm and an emission maximum of 538 nm. The presence of the azide allows the user to use "Click Chemistry" (a [3+2] cycloaddition reaction between alkynes and azides, using copper (I) iodide as a catalyst) to conjugate the TET-Azide to a terminal alkyne-modified oligo with extremely high regionselectivity and efficiency (1,2). Preparation of the alkyne-modified oligo can be achieved using the 5'-Hexynyl modifier (see its respective tech sheet for details). **References** 1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2**: 565-568.

2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

3'

### Oligo Modifications

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#### **Tet-N**

		HOOOOO
Category	Fluorescent Dyes	
Modification Code	Tet-N	a o a
Reference Catalog Number	26-6594	CI
5 Prime	Υ	
3 Prime	Υ	HN A A
Internal	Υ	Pwww Oligo:
Molecular Weight(mw)	675.24	5'-Tetrachloro-Fluorescein (TET) [26-6433-XX]

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Tetrachloro fluorescein (TET) is tetra-chloro derivative of fluorescein that is used to fluorescently label oligonucleotides. TET has an absorbance maximum of 522 nm and an emission maximum of 538 nm. TET plays a role in real-time PCR applications, being used as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, TET is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap.

TET can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with TET at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products. **References** 

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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#### Tide Fluor 5 N

Category Others

Modification Code TF5-N

Reference Catalog Number 26-6604

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 865



#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



Tide Fluor 5 is a fluorescent dye used for labeling oligonucleotides. Tide Fluor 5 has an absorbance maximum of 649 nm and an emission maximum of 664 nm, with spectral properties very similar to those of Cy5. Tide Fluor 5 has very high photostability, and its fluorescence is extremely high and insensitive to pH changes from 3-11. Tide Fluor 5 can be substituted for Cy5 in any FRET-based oligonucleotide assay, for example, TaqMan or Molecular Beacons, or as the reporter dye for fluorescent-based hybridization probes.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

### Oligo Modifications

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#### TXRed-616 N

Category	Fluorescent Dyes		
Modification Code	TXRed-616-N	N O N	
Reference Catalog Number	26-6469		)
5 Prime	Υ	So <sub>3</sub>	TVD   646 11110
3 Prime	Υ		TXRed-616 NHS [26-6469-XX]
Internal	Υ	Υ 0 <i>=</i> S-NH	[20-0-100-701]
Molecular Weight(mw)	406.1	0	O II C-NH/wwoOligo

#### Click here for a list of fluorophores.

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.



TXRed-616 NHS is Sulforhodamine 101 acid chloride, a red-purple fluorescent dye used for labeling oligonucleotides. TXRed-616 NHS has an absorbance maximum of 582 nm and an emission maximum of 600 nm. TXRed-616 NHS can be used in real-time PCR applications as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, TXRed-616 NHS is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

TXRed-616 NHS can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with TXRed-616 NHS at the 5'-end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

TXRed-616 currently is produced in the form of an NHS ester, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The TXRed-616 NHS is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. **References** 

- 1. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 2. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.





Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Oligo Modifications

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#### Yakima Yellow 3'

Category Fluorescent Dyes

Modification Code YYel-3

Reference Catalog Number 26-6700T

5 Prime N

3 Prime Y

Internal N

Molecular Weight(mw) 718.33

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#### Click here for a list of fluorophores.

The Epoch dyes add to the portfolio of the increasing availability of numerous fluorescent dyes covering the visible spectra that suitable for incorporation in oligonucleotides. Yakima Yellow is a substitute for Hex and Vic fluorphores

Single-dye labeled oligonucleotides are routinely used for PCR for fragment analysis, DNA sequencing and FISH analysis. Dual-dye labeled oligos are particularly useful in fluorescence resonance energy transfer (FRET) experiments for determination of intra- and intermolecular distances at very high resolution (1-10 nm). In addition, dual-labeled oligos containing fluorescent dye/dark quencher pairs are now routinely used in quantitative and qualitative real-time PCR experiments and assays (5'-nuclease assay, Molecular Beacon, Scorpions, etc. Details of how such dual-labeled probes work for detection of minute amounts of target are found in the Quenchers modifications category.

Fluorophores can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Fluorescent modifications can also be combined with non-fluorescent modifications in a wide variety of combinations for use in highly specializing applications or research projects.

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# Oligo Modifications

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#### Yakima Yellow 5'

Category Fluorescent Dyes

Modification Code YYel-5

Reference Catalog Number 26-6700

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 718.33

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