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 transcriptase 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. Positive Controls

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.

Dye & Quencher Selection Table

Dye & Quencher Selection Table
References

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ROX (6-Carboxyl-X-Rhodamine) NHS 26-6430
Tamra NHS 26-6450
TAMRA-3' (Carboxytetramethylrhodamine) 26-6451
TAMRA-5' (Carboxytetramethylrhodamine) 26-6947
Tamra-dT (Carboxytetramethylrhodamine-dT) 26-6449
TET (Tetrachloro-Fluorescein) 26-6433
TET Azide 26-6724
Texas Red NHS 26-6469
Tide Fluor 5 Succinimidyl ester 26-6604
Yakima Yellow Epoch 26-6700
2-Amino Purine (2-AP) is a fluorescent molecule that is classified as an adenine and guanine analog, and thus can pair with both thymine and cytosine bases (1). 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 (2). Examples include the hairpin-loop structure of the (CAG)8 repeat, involved in several neurodegenerative disorders—2AP substituted for A (3), the G-quadruplex telomeric structure [AGGG(TTAGGG)3]—2AP substitute for A (4). 2-AP also has been used to characterize the effects of DNA mismatch repair on mutagenesis induced by several different nucleoside analogs (5).

References
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

5. Kurz, M., Gobel, K., Hartel, C., Gobel, M.W. Acridine-labeled primers as tools for the study of nonenzymatic RNA oligomerization.
**Alexa Fluor NHS Ester**

**Category**
-**Fluorescent Dyes**

**Modification Code**
- Alex

**Reference Catalog Number**
- 26-6XXX

**5 Prime**
- Y

**3 Prime**
- Y

**Internal**
- Y

**Molecular Weight (mw)**
- 695.6

Alexa modification is a 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.

Alexa Fluor NHS Esters is a set of fluorescent dyes that span the entire visible electromagnetics 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, Alexa Fluor dyes 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 Alexa Fluor NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes Alexa 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. The molecular structure shown above is for Alexa 488, as an example; note that not all Alexa molecular structures are available. Alexa Fluors are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that Alexa Fluor 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**
Conventional and popular dyes that are derivatives of fluorescein (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 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.
Conventional and popular dyes that are derivatives of fluorescein (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.

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**Product Specifications**

**Oligo Modifications**

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

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<td>Reference Catalog Number</td>
<td>26-6956</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
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<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight (mw)</td>
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Atto 532

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Atto 575Q

Atto 575Q has been replaced by Atto 575Q. 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 (λ 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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**Atto 610**

**Category**

**Modification Code**

Atto610

**Reference Catalog Number**

26-6974

**5 Prime**

Y

**3 Prime**

Y

**Internal**

Y

**Molecular Weight (mw)**

588

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Atto 612Q

Category: Fluorescent Dyes
Modification Code: Atto612Q
Reference Catalog Number: 26-6975
5 Prime: Y
3 Prime: Y
Internal: Y
Molecular Weight (mw): 888

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Atto 633

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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 655

Category     Fluorescent Dyes
Modification Code     Atto655
Reference Catalog Number     26-6981
5 Prime     Y
3 Prime     Y
Internal     Y
Molecular Weight(mw)     887

Conventional and popular dyes that are derivatives of fluorescein (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 designed for high sensitivity applications, including single-molecule detection.

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Atto 700

Conventional and popular dyes that are derivatives of fluorescein (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.

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### Oligo Modifications

**Atto 725**

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<tr>
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<td>Molecular Weight(mw)</td>
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Atto 740

**Category**
Fluorescent Dyes

**Modification Code**
Atto740

**Reference Catalog Number**
26-6987

**5 Prime**
Y

**3 Prime**
Y

**Internal**
Y

**Molecular Weight(mw)**
665

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 designed for high sensitivity applications, including single-molecule detection.

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### Atto Rho101

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<th>Category</th>
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<td>Modification Code</td>
<td>AttoRho101</td>
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<tr>
<td>Reference Catalog Number</td>
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<td>5 Prime</td>
<td>Y</td>
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<td>3 Prime</td>
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<tr>
<td>Internal</td>
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</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>892</td>
</tr>
</tbody>
</table>

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.
Conventional and popular dyes that are derivatives of fluorescein (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 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 Rho14

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Atto Rho3B

Category: Fluorescent Dyes
Modification Code: AttoRho3B
Reference Catalog Number: 26-6965

Structure Not Available

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### Atto Rho6G

**Category:** Fluorescent Dyes  
**Modification Code:** AttoRho6G  
**Reference Catalog Number:** 26-6961

#### Structure Not Available

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.
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 fluorescein (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 designed for high sensitivity applications, including single-molecule detection.

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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.
**CAL Fluor Red 590**

<table>
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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.
CAL Fluor Red 610

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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. 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 an alternative for Texas Red® dye.
CAL Fluor Red 635

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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.
**Oligo Modifications**

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

**Coumarin Azide**

<table>
<thead>
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**Coumarin Azide**

[26-6726-XX]

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**

Cy2 NHS

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<td>Molecular Weight (mw)</td>
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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)).
**Product Specifications**

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.

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**Cy3**

<table>
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<tr>
<td>Reference Catalog Number</td>
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</tr>
</tbody>
</table>

**5' Prime** | **3' Prime** | **Internal** | **Molecular Weight(mw)**  
| Y            | Y             | Y             | 507.6                      |

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 ribosomal 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 microscope 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**

3. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization.
Cy3 NHS modification is a 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 ribosomal 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
2. Thelwell, N., Millington, S., Solinas, A.


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). References
Cy3B modification is a 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 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**

Cy5

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 ribosomal 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.

References
Cy5 disulfo NHS modification is a 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. 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, fluoresceincentred 647 reactive, CF647 succinimidyl ester and PromoFluor ® -647, NHS ester for the required applications.

Reaction scheme for primary amine labelled oligos with NHS ester is shown in the figure below.
Cy5 NHS modification is a 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.

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 uccinimidyl 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 ribosomal 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.

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.


Cy5.5

Cyanine 5.5 (Cy5.5) is a 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). References
Cy7 NHS

Cy7 modification is a 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 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 containing 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

### Oligo Modifications

**Dansyl-X**

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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.
DNP TEG (2, 4-dinitrophenyl)

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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
etheno dA

Category
Modification Code
Reference Catalog Number
5 Prime
3 Prime
Internal
Molecular Weight(mw)

Minor Bases
Eth dA
26-6506
Y
Y
Y
337.23

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
Carcinogenesis (1996), 17: 2105-2111.
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**

Fam-NHS (6-Fam NHS)

Category: Fluorescent Dyes
Modification Code: Fam-N
Reference Catalog Number: 26-6730
5 Prime: Y
3 Prime: Y
Internal: Y
Molecular Weight (mw): 537.46

Fam NHS modification is a 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.

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
FAM-TEG Azide

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
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
3. Ihara, T.
Fluorescein

<table>
<thead>
<tr>
<th>Category</th>
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<tr>
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<td>N</td>
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</table>

Fluorescein is the most commonly used fluorescent dye for labeling oligonucleotides. 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

Fluorescein 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.
**HEX**

**Category**
Fluorescent Dyes

**Modification Code**
HEX

**Reference Catalog Number**
26-6432

**5 Prime**
Y

**3 Prime**
Y

**Internal**
N

**Molecular Weight (mw)**
744.13

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**

**HEX-Azide-6**

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<tr>
<td>Molecular Weight(mw)</td>
<td>665.09</td>
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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**

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.

~500 pmole (0.5 nmol) 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.

References
2. Dar, M., Giesler, T., Richardson, R., Cai, Christine, Cooper, M.
IRDye700

<table>
<thead>
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<td>Reference Catalog Number</td>
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<td>5 Prime</td>
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<td>3 Prime</td>
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<td>Molecular Weight(mw)</td>
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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).

IRDye800

Category: Fluorescent Dyes
Modification Code: IRD800
Reference Catalog Number: 26-6673
5 Prime: Y
3 Prime: Y
Internal: Y
Molecular Weight (mw): 999.3

IRDye800 is a near-IR fluorescent dye used for labeling oligonucleotides. IRDye800 has an absorbance maximum of 778 nm and an emission maximum of 794 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 is used as a reporter moiety in real-time PCR applications. For such probes, IRDye800 is most commonly paired with the dark quencher QC-1, as the two have excellent spectral overlap (2).

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). References

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

**Product Specifications**

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**

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

**LC Red 610**

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<td>5 Prime</td>
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LC Red 610 modification is a 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.

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


**LC Red 640**

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<tr>
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LC Red 640 modification is a 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.

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).

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 610 and Cy5.5, permit LightCycler assays to be in multiplex format. References


Marina Blue

<table>
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<td>Modification Code</td>
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Marina Blue is a proprietary dye manufactured by Life Tech/ThermoFisher, it 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

The amine-reactive Marina Blue® succinimidyl ester can be used to create blue-fluorescent bioconjugates. Based on the 6,8-difluoro-7-hydroxycoumarin fluorophore, Marina Blue® 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. Because the pKa values of Marina Blue® derivatives are 2-3 log units lower than those of the corresponding 7-hydroxycoumarin conjugates, Marina Blue® conjugates are strongly fluorescent even at neutral pH.
Methylene Blue Azide

Category: Redox Electrochemical

Modification Code: MB-N3

Reference Catalog Number: 26-6988

5 Prime: Y
3 Prime: Y
Internal: Y

Molecular Weight (mw): 553

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. Conventional and popular dyes that are derivatives of fluorescein (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 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 equilbrium 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.

**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 have 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 aqueous 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**

Pacific Blue NHS

**Category** | **Fluorescent Dyes**
---|---
**Modification Code** | PcBl
**Reference Catalog Number** | 26-6524
**5 Prime** | Y
**3 Prime** | Y
**Internal** | Y
**Molecular Weight(mw)** | 339.21

Pacific Blue fluorescent dye 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

Pacific Blue is a UV-excitable, bright blue fluorescent dye used for labeling oligonucleotides excitable by the 405 nm spectral line of blue diode (violet color) laser. Pacific Blue has an absorbance maximum of 410 nm and an emission maximum of 455 nm. Because UV light can photodamage labeled oligos, and many kinds of cells and tissues autofluoresce under UV light, Pacific Blue 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, Pacific Blue can be a good choice.
Qdot 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.

As there are a large number of active carboxy groups on the Qdot surface there may be 10 - 80 oligos conjugated to a single Qdot.

- Yield for 50 and 200 nmol scale synthesis is ~20 nmol oligo conjugated to 1 nmol Qdot. Supplied reconstituted as 10 μM Qdot solution in sterile TE pH 7.5. DO NOT FREEZE. STORE at 4 degrees C.
- Yield for 1 umol scale synthesis is ~50 nmol oligo conjugated to 2 nmol of Qdot. Supplied reconstituted as 10 μM Qdot oligo solution in sterile TE pH 7.5. DO NOT FREEZE. STORE at 4 degrees C.

Qdot ITK Carboxyl Quantum Dots (Qdot ITK Carboxyls) are a set of nanometer-scale semiconductor crystalline fluorophores that emit light in the visible and near-IR electromagnetics spectrum (525-800 nm). Qdots are composed of a crystalline CdSe semiconductor core and an outer ZnS semiconductor shell for improved chemical and optical properties. Qdots have several important advantages over traditional organic fluorophore dyes. For organic dyes, the optimal absorption and emission wavelengths are close together. By contrast, for Qdots, the absorption efficiency is shifted far away from and towards the blue (shorter wavelength) side of the emission peak. Because Qdots can be excited at wavelengths far from their emission peak, can inherently absorb more energy in the blue-violet than organic dyes, and can be excited with a single, short-wavelength source irrespective of emission peak, Qdots are much brighter and more photostable, and thus more sensitive, than organic fluorescent dyes. Furthermore, because their emission peaks are significantly narrower than those of organic dyes, Qdots are better suited to applications requiring multiplex detection of several colors simultaneously (1-2).

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Thus, Qdots are unsuitable for any application requiring a 1:1 mole ratio of Qdot:oligo. Qdots are best suited to applications requiring probes in which many oligos are attached to the Qdot surface.

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References
Qdot 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.

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For more information visit ThermoFisher website Qdot Technology Overview.

References
Qdot 605

Qdot 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.

As there are a large number of active carboxy groups on the Qdot surface there may be 10 - 80 oligos conjugated to a single Qdot.

- Yield for 50 and 200 nmol scale synthesis is ~20 nmol oligo conjugated to 1 nmol Qdot. Supplied reconstituted as 10 μM Qdot solution in sterile TE pH 7.5. DO NOT FREEZE. STORE at 4 degrees C.
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- Yield for 50 and 200 nmol scale synthesis is ~20 nmol oligo conjugated to 1 nmol Qdot. Supplied reconstituted as 10 μM Qdot solution in sterile TE pH 7.5. DO NOT FREEZE. STORE at 4 degrees C.
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References
Qdot 705

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As there are a large number of active carboxy groups on the Qdot surface there may be 10 - 80 oligos conjugated to a single Qdot.

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Qdot 800

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References
Quasar 670

Quasar 670 is a dye manufactured by Biosearch Technologies, it 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 compound 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 Quasar 670 is BBQ650 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

<table>
<thead>
<tr>
<th>Quencher</th>
<th>Absorption Max, nm</th>
<th>Quenching Range, nm</th>
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<tr>
<td>Dabcyl</td>
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<td>BHQ1</td>
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<td>BBQ-650</td>
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Click here for complete list of quenchers **Black Hole Quencher License Agreement**

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Rhodamine Red X

**Category**  | **Fluorescent Dyes**  
---|---
Modification Code  | RhoR-XN  
Reference Catalog Number  | 26-6606  
5 Prime  | Y  
3 Prime  | Y  
Internal  | Y  
Molecular Weight (mw)  | 768.9

Rhodamine Red 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

Rhodamine Red X is a red fluorescent dye used for labeling oligonucleotides excitable by the 568 nm spectral line of the krypton/argon ion laser. Rhodamine Red X has an absorbance maximum of 570 nm and an emission maximum of 590 nm. Rhodamine Red X can be used to label oligos slated for typical molecular biology applications (RT-PCR, hybridization probes, etc). Rhodamine Red X is particularly useful in experiments requiring triple labeling along with Cy2 and Cy5 because Rhodamine Red X’s emission maximum lies midway between those of Cy2 and Cy5 and its spectrum has little overlap with these other fluorophores.
ROX modification is a 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.

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. References

# Tamra NHS

**Category**  
**Fluorescent Dyes**

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<tr>
<th>Modification Code</th>
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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.
Tamra-3’

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
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References
Tamra-dT

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<th>Category</th>
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<tbody>
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<td>5 Prime</td>
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<td>Molecular Weight(mw)</td>
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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.
TET

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

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

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 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). 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.
Sulforhodamine 101 acid chloride (Texas Red) is a red-purple fluorescent dye used for labeling oligonucleotides. Texas Red has an absorbance maximum of 589 nm and an emission maximum of 615 nm. Texas Red 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, Texas Red is most commonly paired with the dark quencher BHQ-2, as the two have excellent spectral overlap.

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

Tide Fluor 5

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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.
**Yakima Yellow**

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</tbody>
</table>

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See detailed license restrictions and provisions below.

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 fluorophores

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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