



Product Specifications

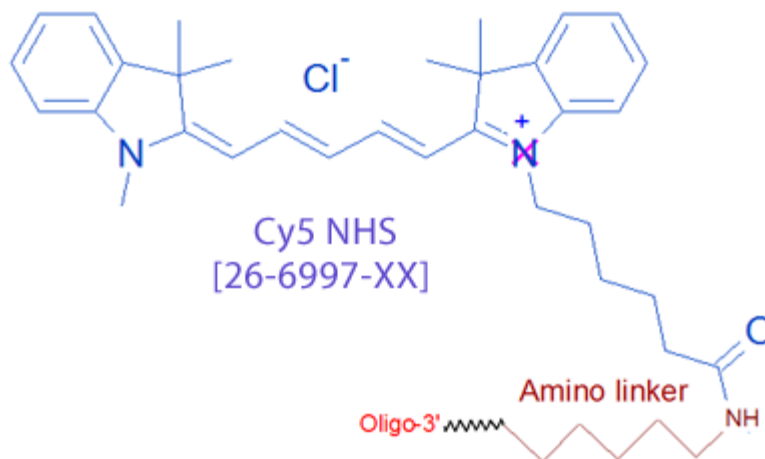
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

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

Cy5 NHS

Category	Fluorescent Dyes
Modification Code	Cy5-N
Reference Catalog Number	26-6997
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	537



Click here for a list of fluorophores.

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

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

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

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

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

Cy5 can also be used to label DNA oligos for use as hybridization probes in other applications, such as Fluorescent In-Situ Hybridization (FISH). In 2010, Stoeckler and co-workers (4) reported that Cy5 double-labeling of FISH probes (at both ends) that were specific to 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.

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Absorbance Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient*

Color**

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800

AZ680 NHS 678 701 185,000

Cy5.5 684 710 198,000

IRDye 700 NHS 684 710 288,000

AZdye700 NHS 696 719 192,000

Atto 700 NHS 700 716 120,000

Atto 725 NHS 728 751 120,000

Atto 740 NHS 743 763 120,000

Cy7 NHS 740 773 199,000

IRDye 750 NHS 756 776 260,000

cy7.5 NHS 788 808 223,000

IRDye 800 NHS 795 819 240,000

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

[Click here for a list of fluorophores.](#)

[Click here for list of quenchers.](#)

References

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

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

