Product Specifications

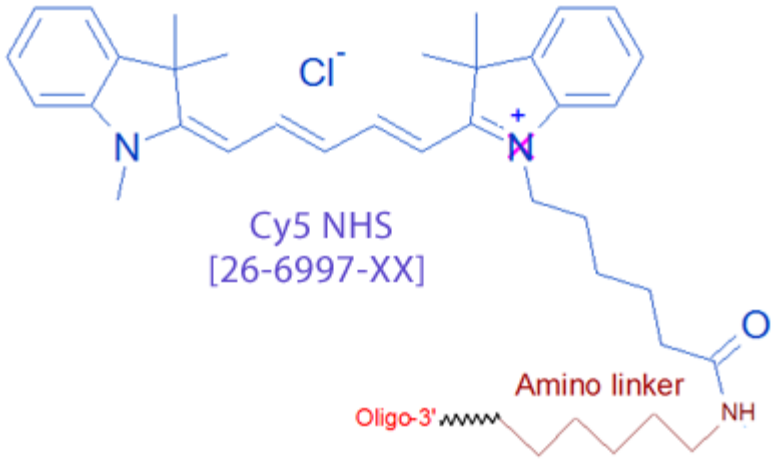
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2′-5′ linked Oligos

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



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. **YIELD** NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis

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

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

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

Near Infrared Fluorophore Spectral Data & Quencher Selection Guide

Fluorophore Name

Excitation Max, nm +/-10

Emission Max, nm +/-10

Extinction Coefficient*

Color**

Quencher

Cy5 650 665 250,000

IRDye 650 NHS 650 665 230,000

AZ647 NHS 655 680 191,800

Cy5.5 684 710 198,000

IRDye 700 NHS 684 710 288,000

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

Click here for a list of fluorophores.

Click here for list of quenchers.

References

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

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

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

4. Stoecker, K., Dorninger, C., Daims, H., Wagner, M. Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) Improves Signal Intensity and Increases rRNA Accessibility. *Appl. Environ. Microb..* (2010), 76: 922-926.

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

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