



## Product Specifications

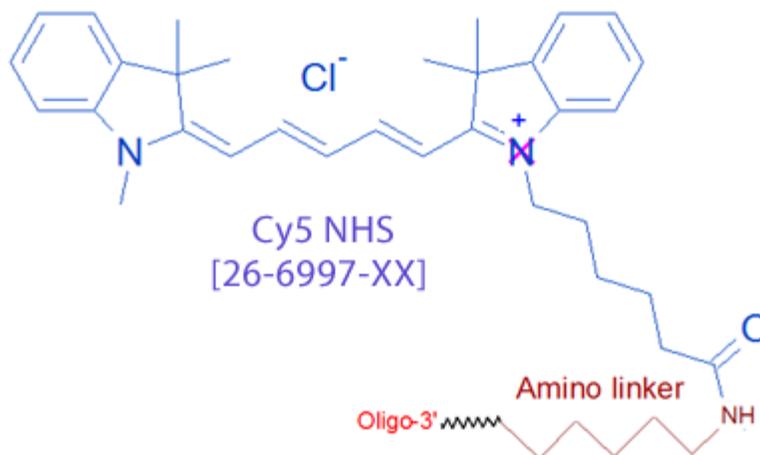
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## 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.

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

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

#### - References

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

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

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.