



## Product Specifications

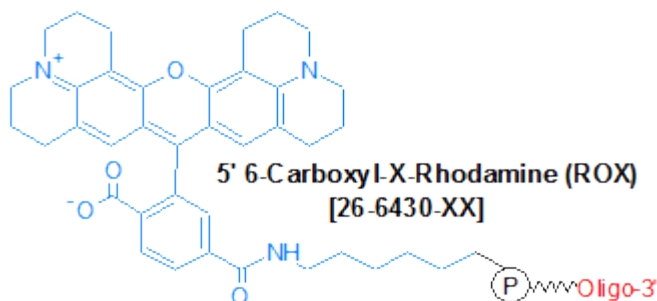
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### ROX

Category	Fluorescent Dyes
Modification Code	ROX-N
Reference Catalog Number	26-6430
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	516.6



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. **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

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

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

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

Applied Biosystems Proprietary Dyes & Possible Substitutions

Dye

Color

Absorbance max (nm)

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

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

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

#### **References**

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