



## Product Specifications

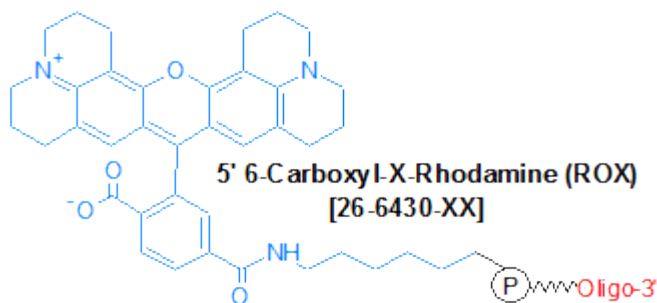
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### ROX N

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



**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.

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.