### Oligo Modifications

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Fluorescent Dyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>6-ROXN</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6430</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
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<tr>
<td>Internal</td>
<td>Y</td>
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<tr>
<td>Molecular Weight(mw)</td>
<td>550</td>
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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. References