ROX™ Passive Reference Dye

Product number 75768

Brief protocol

**ROX™ spectral characteristics**

<table>
<thead>
<tr>
<th>Excitation Maximum λ</th>
<th>≈ 575 nm</th>
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</thead>
<tbody>
<tr>
<td>Emission Maximum λ</td>
<td>≈ 600 nm</td>
</tr>
</tbody>
</table>

ROX Passive Reference Dye is specially formulated for use on Applied Biosystems™ (ABI) and Stratagene™ real-time PCR instruments. This inert dye, whose fluorescence does not change during the reaction, may be added to quantitative, real-time PCR reactions to normalize the well-to-well differences that may occur due to artifacts such as pipetting errors or instrument limitations.

ROX Passive Reference Dye is composed of a 25 μM solution of 5-carboxy-X-rhodamine in 10 mM Tris-HCl (pH 8.6), 0.1 mM EDTA, and 0.01% Tween™-20. Although many instruments require use of the dye at final concentrations of 500 nM, newer instruments with optimized filter sets require use at 50 nM. See specific instrument instructions for further details on passive dye usage. The following table shows current recommendations:

**ROX™ final concentration for different instruments**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Amount per 50 μl reaction</th>
<th>Final ROX concentration</th>
<th>Dilution factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI 7000, 7300, 7700, 7900HT, 7900HT Fast, and StepOnePlus™</td>
<td>1.0 μL (0.6-1.0 μL)</td>
<td>500 nM (300-500 nM)</td>
<td>50X</td>
</tr>
<tr>
<td>ABI 7500 and ABI 7500 Fast; Stratagene Mx3000™, Mx3005P™, and Mx4000™</td>
<td>0.1 μL (0.06-0.1 μL)</td>
<td>50 nM (30-50 nM)</td>
<td>500X</td>
</tr>
</tbody>
</table>

Subtract the volume of the dye from the volume of water needed to prepare the PCR reaction.

*Note: ROX may be diluted 1:10 in water to aid in accurate pipetting.
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  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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