

# ROX™ Passive Reference Dye

**Product number 75768**

## Brief protocol

### ROX™ spectral characteristics

Excitation Maximum $\lambda$	≈ 575 nm
Emission Maximum $\lambda$	≈ 600 nm

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  $\mu$ M 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

Instrument	Amount per 50 $\mu$ l reaction	Final ROX concentration	Dilution factor
ABI 7000, 7300, 7700, 7900HT, 7900HT Fast, and StepOnePlus™	1.0 $\mu$ L (0.6-1.0 $\mu$ L)	500 nM (300-500 nM)	50X
ABI 7500 and ABI 7500 Fast; Stratagene Mx3000™, Mx3005P™, and Mx4000™	0.1 $\mu$ L (0.06-0.1 $\mu$ L)*	50 nM (30-50 nM)	500X

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.

## Documentation and support

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- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - 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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