

# ROX Passive Reference Dye

172-5858

0.5 ml

For research purposes only.  
Store at  $-20^{\circ}\text{C}$ , protect from light.

ROX passive reference dye is a stabilized conjugate of carboxy-X-rhodamine in water. The emission property of this dye has been optimized to allow seamless integration of non-ROX-containing amplification reagents on ABI PRISM sequence detection systems, and is compatible with the default pure dye spectra for ROX. Many real-time thermal cyclers require an internal reference dye for normalization of fluorescent signal and correction of well-to-well optical variations. Since excitation optics vary on different instrument platforms, the optimal reference dye concentration must be matched to a given real-time thermal cycler.

For the ABI PRISM 7000, 7300, 7700 or 7900 instruments, the ROX passive reference dye should be used directly as a 50x concentrated solution — simply include 1  $\mu\text{l}$  of ROX passive reference dye in each 50  $\mu\text{l}$  real-time PCR reaction. As with any real-time PCR procedure, preparation of a mastermix is critical to ensure uniform distribution of reagent(s) to all reactions.

For instruments employing a 580–585 nm excitation for the passive reference, such as the Stratagene Mx4000, Mx3000P, Mx3005P, or ABI PRISM 7500 sequence detection system, the ROX passive reference dye should be treated as a 750x concentrated solution. The dye may be diluted 1:15 in 10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA and then used as a 50x concentrated solution as described above. Alternately, one can prepare a "ready-to-use" 2x supermix by adding 3.3  $\mu\text{l}$  of ROX passive reference dye to 1.25 ml of 2x iQ™ supermix or the 2x RT-PCR reaction mix component of the iScript™ one-step RT-PCR kits.

Use of an internal reference is not required when using the Bio-Rad iQ™, MyiQ™, MJ Research® Opticon™, DNA Engine Opticon® 2, or Chromo4™ real-time thermal cyclers and systems.

## **Storage and Stability**

ROX passive reference dye is stable for 1 year when stored in a constant temperature freezer at  $-20^{\circ}\text{C}$ . For convenience, it may be stored unfrozen at  $2-8^{\circ}\text{C}$  for up to 6 months. After thawing, mix thoroughly before using.

## Related Amplification Products from Bio-Rad

Reagents for PCR or real-time PCR	iTaq™ DNA Polymerase, 170-8870 iQ™ Supermix, 170-8864 iQ SYBR® Green Supermix, 170-8884 iTaq™ Supermix with ROX, 172-5856 iTaq SYBR® Green Supermix with ROX, 172-5852 iScript™ cDNA Synthesis Kit, 170-8891 iScript Select cDNA Synthesis Kit, 170-8897 iScript One-Step RT-PCR Kit for Probes, 170-8895 iScript One-Step RT-PCR Kit with SYBR® Green, 170-8893
Other reagents	dNTP Mix, 200 µl, 10 mM each dNTP, 170-8874 MgCl <sub>2</sub> Solution, 50 mM, 1.25 ml, 170-8872
Pipet tips, aerosol barrier tips	Xcluda® Style B, 211-2006
Nuclease-free tubes and plates	0.2 ml Thin-Wall Tubes, 223-9473, or plates, 223-9441
RNA purification kit	Aurum™ Total RNA Mini Kit, 732-6820, or Aurum Total RNA Kit, 2 x 96 well, 732-6800

To learn more about Bio-Rad's complete solution for amplification, visit our website:  
[www.bio-rad.com/amplification](http://www.bio-rad.com/amplification)

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