ROX染料(50 倍浓度)

Description

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 is a reference dye which will help you calibration the initial fluoresence reading of every tube.

For most machine, before you run any real-time PCR, the camera will try detect the presence of particular dye and normalised the flouresence reading of every single tube. This is to make sure that your every tube start reaction at the same flouresence unit.

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

ROX™ Final Concentration for Different Instruments:

ABI 7000, 7300, 7700, 7900HT and 7900HT Fast:

Amount per 50 μl reaction: 1.0 μl (0.6-1.0 μl)

Final ROX Concentration: 500nM (300-500nM)

Dilution Factor: 50X

ABI 7500 and ABI 7500 Fast; Stratagene Mx3000™, Mx3005P™, and Mx4000™:

Amount per 50 μl reaction: 0.1 μl (0.06-0.1 μl)*

Final ROX Concentration: 50nM (30-50nM)

Dilution Factor: 500X

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

Spectral Characteristics:

Excitation Maximum : ≈ 575 nm

Emission Maximum : ≈ 600 nm

Storage:

Shipped on ice bag. Store at -20°C. Protect from light.

Reaction Mixture Set Up for qPCR

1.Gently vortex and briefly centrifuge all solutions after thawing.

2.Add, in a thin-walled PCR tube, on ice:

Reagent	Quantity, for 50µl of reaction mixture	Final concentration
Sterile deionized water	variable	-
10X *Taq* buffer	5µl	1X
10mM dNTP mix	1µl	0.2mM of each
25mM $MgCl_2$	7ul	3.0-4.0mM
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
Taqman probe/Sybr Green I	variable	0.2-0.3uM/0.2 X
Taq DNA Polymerase	0.5	2.5u / 50µl
50 X *ROX*	1	1
Template DNA	variable	10pg-1µg
Total	50ul	

Cycling Protocol for qPCR

1、Protocol using LightCycler with Taqman probe

93℃ 2min→93℃5 sec→60℃30 sec

40cyclers

2、Protocol using LightCycler with Molecular Beacon probe

93℃ 2min→93℃5 sec→60℃20 sec→72℃20 sec

40cyclers

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3、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Taqman probe

94℃ 4min→94℃ 30sec→60℃ 60sec
40cyclers

4、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Molecular Beacon probe

94℃ 4min→94℃ 30sec→60℃ 30sec→72℃30sec
40cyclers

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