HotRox Master Mix, high Rox

Description

HotRox Master Mixes of BIORON are optimized ready-to-use mixes for the amplification and detection of DNA in Rreal Tme quantitative PCR (qPCR). They contain all necessary components to perform quantitative PCR, with the exception of template and primers. ROX reference dye is included in the HotRox Master Mixes to normalize the fluorescent signal on instruments that are compatible with this option. The HotRox Master Mix contains SuperHotTaq polymerase of BIORON supplied in a proprietary reaction buffer that enables detection of low copy number targets.

Content

HotRox Master Mix (2x) (ROX 1 µM, $MgCl_2$ 3.0 mM)	**Sample size**	**200 reactions**	**1000 reactions**
Cat. No.	**S119550**	**119550**	**119552**
HotRox Master Mix (2x) *	**1x 300µl**	**2x 1.25 ml**	**10x 1.25 ml**
$MgCl_2$ 100 mM	**1 ml**	**1ml**	**5x 1ml**
PCR Water	**1.8 ml**	**2x 1.8 ml**	**10x 1.8 ml**
Datasheet	**1**	**1**	**1**

***Contains Antibody blocked Hotstart Taq DNA Polymerase (recombinant), PCR Buffer with 3 mM $MgCl_2$,**
400 µM each dNTP and 1µM ROX reference dye.

Instrument	**Final ROX Conc.**
ABI 7000, 7300, 7700, 7900HT	500 nM

Features

- High sensitivity & specificity
- SuperHotTaq DNA Polymerase of BIORON included (Taq Polymerase with antibodies versus Taq polymerase)
- ROX reference dye (1 µM) is included to normalize the fluorescent signal on instruments that are compatible with this option.

Applications

- Real Time qPCR assays
- End point analysis

Storage condition

Store at -20 °C. Protect from light. Avoid repeated freezing/thawing. Shipped on blue-ice.

Protocol for PCR with HotRox Master Mix

Due to the inhibition of polymerase activity at room temperature by Anti Taq DNA polymerase antibodies all reactions can be settled-up at room temperature, it will not result in an increase of unspecific product or primer-dimer formation.

HotRox Master Mix is twofold concentrated 2 × 1.25 ml is enough for 200 rcs with a final reaction volume of 25 µl.

Add in a thin walled PCR tube:

25 µl reaction volume		
Component	**Volume**	**Final concentration**
2x HotRox Master Mix	12.5 µl	1x
Forward Primer	variable	0.1-1 µM
Reverse Primer	variable	0.1-1 µM
Template DNA	variable	10 pg-1 µg
Sterile deionized water	up to 25 µl	-

- Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.
- Place the samples in a thermocycler and start a PCR program

Real-time PCR amplification is done on ABI®PRISM 7700, iQ5™ Real Time PCR System (BioRad) or other appropriate machines for Real-time PCR suitable for use of intercalating dye chemistry. All samples are run in triplicate with the appropriate single PCR controls (no template, no primers). Always prepare 2 Master Mixes for gene of interest and control gene to be sure in experiment-to-experiment consistency.

Real-Time Cycler Conditions

step	**time**	**temperature**
initial denaturation	2 minutes	94 °C
30 cycles:		
denaturation	10 seconds	94 °C
annealing	20 seconds	55 - 68 °C *
extension	1 minute	72 °C

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers

Notes:
Program the cycler according to the manufacturers instructions. Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.
Recommended elongation time is 1 min per 1 kb of target.
For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.