HotRox Master Mix, low Rox

Description HotRox Master Mixes of BIORON are optimized ready-to-use mixes for the amplification and detection of DNA in Real Time 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. The HotRox Master Mix contains SuperHotTaq polymerase of BIORON supplied in a proprietary reaction buffer that enables detection of low copy number targets.

Content

Ref No.	S119505	119505	color
HotRox Master Mix 2x * (ROX 0.1 µM)	**Sample size**	**200 reactions**	**brown tube**
$MgCl_2$ 100 mM	**1 mL**	**1 mL**	**green**
PCR Water	**1.8 mL**	**2x 1.8 mL**	**transparent**
Datasheet	**1**	**1**	**--**

* Contains Antibody blocked Hotstart Taq DNA Polymerase (recombinant), PCR Buffer with 3 mM $MgCl_2$, 400 µM each dNTP and 0.1 µM ROX reference dye.

Applications: HotRox Master Mixes are suitable for qPCR and point analysis. ROX reference dye (0.1 µM) is included to normalize the fluorescent signal on instruments that are compatible. The use of HotRox Master Mix with low Rox or high Rox concentrations depends on the used instrument.

Instrument	Final ROX Conc.
ABI 7500™; Agilent™ Mx3000, MX3005P, Mx4000; QuantStudio™	50 nM

Concentration: 2x HotRox Master Mix with 0.1 µM ROX

Sensitivity: high

Unit Definition: --

Additionally provided: 1 tube $MgCl_2$ (100 mM)

Quality Control

- No detectable exo-/endonuclease activities
- PCR amplification tests with different templates
- PCR amplification tests without templates as Negative Control
- Hotstart efficiency test showing effective blockage by AntiTaq

Storage conditions: -20 °C

BIORON GmbH – In den Rauhweiden 20 - 67354 Römerberg (Germany)
Phone +49 6232 298 45 0 - Fax +49 6232 298 45 29
info@bioron.net www.bioron.net

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Protocol for PCR with HotRox Master Mix

HotRox Master Mix is twofold concentrated 2 × 1.25 mL is enough for 200 reactions with a final reaction volume of 25 µL.

Add in a thin walled PCR tube:

Components	Volume / 25 µL PCR-Reaction	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 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 manufacturer's 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.

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