



The ENZYME Company

HotRox Master Mix 2x

Cat.-No.: 119505 100 rxn

Description

2x HotRox Master Mix is an optimized ready-to-use PCR mixture of *Taq* DNA Polymerase, antibodies to *Taq* DNA polymerase, PCR buffer, MgCl₂ and dNTPs. 2x PCR Master Mix contain all components for PCR, except DNA template and primers. ROX Reference dye is included in the HotRox Master Mix to normalize the fluorescent signal on instruments that are compatible with this option. The mixture was shown to be effective for Real Time PCR.

2x HotRox Master Mix Composition

- *Taq* DNA Polymerase (recombinant) in reaction buffer: 0.1 units/μl
- Antibodies to *Taq* DNA polymerase, concentration adjusted for the effective inhibition of polymerase activity at 37°C
- 32 mM (NH₄)₂SO₄
- 130mM TrisHCl, pH 8.8 at 25°C
- 0,02% Tween-20
- 3 mM MgCl₂
- dNTPs (dATP, dCTP, dGTP, dTTP): 0.4mM of each
- ROX Reference dye (0,1μM)

Instrument	Final ROX Conc.
ABI 7000, 7300, 7700, 7900HT	500 nM
ABI 7500; Stratagene Mx3000, MX3005P, Mx4000	50 nM

Pack size 2x 1,25ml – sufficient for 100 HotStart PCR reactions in 50 μl reaction volume. **Supplied with the Mixes:** 1ml tube MgCL₂ (100mM)

Performance and purity tests

Tested for the absence of endodeoxyribonucleases and exodeoxyribonucleases. The 2x HotRox Master Mix is tested in the amplification of a single-copy gene of mouse genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 25μl of 2x HotRox Master Mix with 1μg of pUC19 DNA in 50μl for 4 hours neither at 37°C nor at 70°C

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 may be setted-up at room temperature, it will not result in increase of unspecific product or primer-dimers formation.

Add in a thin walled PCR tube:

50μl reaction volume:			25μl reaction volume:	
Component	Volume	Final concentration	Volume	Final concentration
2X PCR Master Mix	25μl	1X	12.5μl	1X
Forward Primer	variable	0.1-1μM	variable	0.1-1μM
Reverse Primer	variable	0.1-1μM	variable	0.1-1μM
Template DNA	variable	10pg-1μg	variable	10pg-1μg
Sterile Deionized Water	up to 50μl	-	up to 25μl	-

- Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.
- Overlay the sample with mineral oil or add an appropriate amount of wax if the thermal cycler is not equipped with a heated lid.

- Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.

Bioron

Contact Germany: Phone: +49-(0)-621- 5720 915

Contact Singapore Phone: +65 6896 6942

E-Mail: info@bioron.net WEB: www.bioron.net



The ENZYME Company

- Place the sample in a thermocycler and start a PCR program.

Catalog #	Description	Pack size
119550	HotRox Master Mix 2x (Final ROX conc. 500nM)	100 rcs
119505	HotRox Master Mix 2x (Final ROX conc. 50nM)	100 rcs

Version 1.2 05.04.2007AS

Bioron

Contact Germany: Phone: +49-(0)-621- 5720 915
Contact Singapore Phone: +65 6896 6942
E-Mail: info@bioron.net WEB: www.bioron.net