

Real-time-PCR Master Mix EVA1 (2x) for High Resolution Melt (HRM) - Technics
Cat.-No: S150, 2x1,25 ml

Features:

- The Master mix contains dTTP
- It contains EvaGreen as fluorescent dye
- The qPCR / RT-PCR Mastermix EVA 1 is ready-to-use and is optimized for high specificity and sensitivity because of optimized reaction buffer
- Easy to use because ready-to-use Master Mix
- The Master Mix can be used with ROX as reference dye (1x concentrated)

Applications:

- Detection and quantification of DNA and cDNA targets
- Profiling gene expression
- Microbial detection
- Viral load determination
- Analysis of SNPs, mutations and methylations in PCR amplicons

Description:

The Master Mix contains all reagents required for qPCR (except template and primer) in a premixed 2x concentrated ready-to-use solution. The high specificity and sensitivity of the mix is achieved by an optimized hot-start polymerase. Its activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of non-specifically annealed primers and primer-dimer formations at low temperatures during PCR setup.

Concentration: The Master mix is 2x concentrated

List of components:

Hot-Start Polymerase for qPCR, dATP, dCTP, dGTP, dTTP (0,5mM each), optimized reaction buffer with EvaGreen and 4mM MgCl₂, stabilizers and enhancers, PCR-grade water

Transportation: with blue ice

Storage: at 4°C for 3 months, at -20°C for 16 months, Note: protect from Light

Usage:

Components	Volume per reaction	final conc.
2X qPCR-Master mix EVA1	25 µl	1x
Up-stream primer (10 µM stock)	1,5 µl (range: 0,5-2.5 µl)	300 nM
Down-stream primer (10µM stock)	1,5 µl (range: 0.5-2,5 µl)	300 nM
Template DNA	5 µl (0.1-15 ng/ml plasmid DNA) (1-10 µg/ml genomic DNA)	< 500ng DNA
Sterile dest. Water (included)	up to 50 µl total reaction volume	

- vortex all solutions carefully before using and before PCR
- may you add the enzyme mix after Template DNA
- an individual optimization of annealing temperature may be necessary for new combinations of primers and Template DNA

.. a good decision ..

General Thermo-Cycler protocol for qPCR-Master mix:

Note: working with EvaGreen just select the optical setting for FAM or SYBR Green at the cycler

Step	Time	Temperature
UNG treatment optional	2 min	50°C
Initial denaturation	2-3 min	95°C
25-45 Cycles:		
Denaturation	15-30 sec	95°C
Annealing	30-65 sec	52-68°C
Extension	20-60 sec (per 500bp)	72-74°C

Note:

- an individual optimization of annealing temperature may be necessary for new combinations of primers and Template DNA
- up to 10x repeated freeze-thaw cycles do not influence the activity of this product

Recommended ROX mixture:

HighROX (0.6-1 µl per 50 µl of total reaction volume): Applied Biosystems: 7000, 7300, 7700, 7900HT Fast, StepOne, StepOnePlus.

LowROX (0.6-1 µl per 50 µl of total reaction volume): Applied Biosystems: 7500, Stratagene: Mx3000P, Mx3005P, Mx4000P.

Ordering information:

Cat.-no	Description	Amount
S150	Real-time-PCR Mastermix EVA1 (2,5 ml)	200 rcs / 25µl
S150L	Real-time-PCR Mastermix EVA1 (25 ml)	2000 rcs / 25 µl

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