

**Real-time-PCR Master Mix EVA4 (2x) for Genotyping and High Resolution Melt (HRM)**  
**Cat.-No: S180, 2x1,25 ml**

**Features:**

- The Master mix contains dUTP, UNG and HIGH-ROX
- It contains EvaGreen as fluorescent dye
- The qPCR Mastermix EVA4 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 contains ROX as reference dye

**Applications:**

- Detection and quantification of DNA and cDNA targets
- Profiling gene expression
- Microbial detection
- Viral load determination

**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.

The mix contains dUTP instead of dTTP and allows an UNG (Uracil-N-Glykosylase) treatment at the onset of thermal cycling to prevent carry-over contaminations of DNA from previous PCR reactions.

**Concentration:** The Master mix is 2x concentrated

**List of components:**

Hot-Start Polymerase with monoclonal antibodies for qPCR, dATP, dCTP, dGTP, dUTP, UNG, EvaGreen, HIGH-ROX (1µM), optimized reaction buffer with KCl, ammonium sulfate and MgCl<sub>2</sub>, 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.
<b>2X PCR Master mix EVA4</b>	25 µl	1x
<b>Up-stream primer (10 µM stock)</b>	1,5 µl (range: 0,5-2.5 µl)	300 nM
<b>Down-stream primer (10µM stock)</b>	1,5 µl (range: 0.5-2,5 µl)	300 nM
<b>Template DNA</b>	5 µl (0.1-15 ng/ml plasmid DNA) (1-10 µg/ml genomic DNA)	< 500ng DNA
<b>Sterile dest. Water (included)</b>	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 ..*

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**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	2 min	50°C
Initial denaturation	1-3 min	95°C
<b>30-40 Cycles:</b>		
Denaturation	15-30 sec	95°C
Annealing	30-65 sec	55-65°C
Extension	30 sec (per 500bp)	72-75°C

**Note:**

- an individual optimization of annealing temperature may be necessary for new combinations of primers and Template DNA

**Ordering information:**

Cat.-no	Description	Amount
S180	Real-time-PCR Mastermix EVA4 (2,5 ml)	200 rcs / 25µl
S180L	Real-time-PCR Mastermix EVA4 (12,5 ml)	1000 rcs / 25 µl

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