

## Real-time-PCR Master Mix E4

### Features:

- The Master mix contains dUTP instead of dTTP
- The Master contains UNG (Uracil-N-Glycosylase)
- The Mix contains ROX (500nM) as passive Reference dye (it provides a baseline in multiplex reactions)
- It contains EvaGreen as fluorescent dye
- The qPCR / RTD-PCR Master mix E4 is ready-to-use and is optimized for high specificity and sensitivity because of optimized reaction buffer
- easy to us because ready-to-use Master Mix

### 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 offer dUTP instead of dTTP to prevent carry-over contaminations of DNA from previous PCR reactions.

**Concentration:** The Mastermix is 2x concentrated

### List of components qPCR / RTD-PCR Master mix:

Hot-Start Polymerase for qPCR, dATP, dCTP, dGTP, dUTP, EvaGreen, ROX, UNG, optimized reaction buffer with KCl 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 more than 12 months, **Note:** protect from Light

### Usage:

Components	Volume per reaction	final conc.
<b>2X qPCR / RTD-PCR Master mix E4, with UNG</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

**General Thermo-Cycler protocol:**

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

Step	Time	Temperature
UNG treatment	1x2 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	qPCR / Real-time PCR Mastermix E4	100 rcs / 50µl