

Real-time-PCR Master Mix E2 Cat-No: S160, 100 rcs

Features:

- The Master mix contains dUTP instead of dTTP
- The Master contains UNG (Uracil-N-Glycosylase)
- It contains EvaGreen as fluorescent dye
- The qPCR / RTD-PCR Master mix E2 is ready-to-use and is optimized for high specificity <u>and</u> sensitivity because of optimized reaction buffer
- easy to us 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

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. It contains optimized UNG (Uracil-N-Glycosylase) for the treatment at the onset of thermal cycling.

Concentration: The Mastermix is 2x concentrated

List of components qPCR / RTD-PCR Master mix:

Hot-Start Polymerase for qPCR, dATP, dCTP, dGTP, dUTP, EvaGreen, optimized reaction buffer with KCl and MgCl₂, Uracil-N-Glycosylase, 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.
2X qPCR / RTD-PCR Master mix E2, with UNG	25 μΙ	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



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
30-40 Cycles: Denaturation Annealing Extension	15-30 sec 30-65 sec 30 sec (per 500bp)	95°C 55-65°C 72-75°C

Note:

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

Ordering information:

Catno	Description	Amount
S160	Real-time PCR Master mix E2	100 rcs / 50µl