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Features:

- The Master mix contains dUTP instead of dTTP
- The Mix contains ROX (500nM; LOW-ROX-version 100mM) as passive Reference dye (it provides a baseline in multiplex reactions)
- It contains EvaGreen as fluorescent dye
- The qPCR / RTD-PCR Master mix E3 is ready-to-use and is optimized for high specificity <u>and</u> sensitivity because of optimized reaction buffer
- easy to use because ready-to-use Master Mix

Compatibility:

500 nM Version: ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus **100 nM Version**: 7500 System, Stratagene Mx35005P, Mx4000 and Mx3000P, ViiA 7, QuantStudio

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, optimized reaction buffer with KCl and MgCl₂, stabilizers and enhancers, PCR-grade water

Transportation: with dry ice

Storage: at 4°C for 3 months, at -20°C for more than 12 months,

Note: protect from Light



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Usage:

| Components | Volume per reaction | final conc. |
|------------------------------------|--|-------------|
| 2X qPCR / RTD-PCR Master mix E3/E5 | 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 | 15-30 sec | 95°C |
| Annealing | 30-65 sec | 55-65°C |
| Extension | 30 sec | 72-75°C |

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

Order Information

| Prod. No. | Description | Quantity |
|-----------|-----------------------------------|------------------|
| S9170 | qPCR / real-time PCR Mastermix E3 | 100 rcs (2.5ml) |
| S9170L | qPCR / real-time PCR Mastermix E3 | 500 rcs (12.5ml) |
| S9170XL | qPCR / real-time PCR Mastermix E5 | 1000 rcs (25mll) |

