



qPCR Multiplex Mastermix (2x)

Features:

- optimized realtime PCR Mastermix using probe based detection (e.g. FRET, Molecular Beacons or TaqMan)
- The Master mix contains dUTP instead of dTTP
- The qPCR Multiplex Mastermix 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 for block based PCR Cycler
- 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

Available mixtures:

- NO-ROX (product code: M100-200; M100-1000)
- LOW-ROX (product Code: M110-200, M110-1000)
- HIGH-ROX: (product code: M120-200, M120-1000)

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.

Multiplex assays with ≥ 4 target sequences in a single tube.

Concentration: The MultiplexMaster mix is 2x concentrated

List of components:

Hot-Start Polymerase for qPCR, dATP, dCTP, dGTP, dUTP, optimized reaction buffer with KCl and MgCl₂, stabilizers and enhancers, PCR-grade water

Transportation: with blue ice

Storage: at 4°C for 3 months, at -20°C for more than 16 months

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Usage:

Components	Volume per reaction	final conc.
2X qPCR / RTD-PCR Master mix DLP1	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
- optimal primer concentration may vary from 100 – 500 nm
- optimal primer concentration may requires titration and should be getween 50-500nM

Note: Best is to prepare 13 reactions of Mastermixes for 12 samples

General Thermo-Cycler protocol for qPCR / RTD-PCR Master mix:

Step	Time	Temperature
Initial denaturation	1-3 min	95°C
35-50 Cycles:		
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
M100-200	qPCR MultiplexMastermix (2x) (no ROX)	2x1,25 ml
M100-1000	qPCR MultiplexMastermix (2x) (no ROX)	10x1,25 ml
M110-200	qPCR MultiplexMastermix (2x) (Low ROX)	2x1,25 ml
M110-1000	qPCR MultiplexMastermix (2x) (Low ROX)	10x1,25 ml
M120-200	qPCR MultiplexMastermix (2x) (High ROX)	2x1,25 ml

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M110-100	qPCR MultiplexMastermix (2x) (High ROX)	10x1,25 ml
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