

qPCR ProbesMaster with UNG / ROX

Master mix for quantitative real-time PCR using labeled DNA probes

Real-Time PCR

| Cat.-No. | Amount |
|----------|-----------------------|
| PCR-302S | 100 reactions x 50 µl |
| PCR-302L | 500 reactions x 50 µl |

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20°C, avoid frequent thawing and freezing

Storage at 4°C for up to 3 months possible

qPCR Master with UNG/ROX must be stored in the dark

Description

qPCR ProbesMaster with UNG / ROX is designed for the quantitative real-time analysis of DNA samples using DNA probe based detection. The master mix is recommended for use with Dual Labeled Fluorescent Probes, e.g. TaqMan[®], Molecular Beacons or FRET probes. It provides an easy-to-handle and powerful tool for quantification of sample DNA in a broad dynamic range of up to 6 orders of magnitude with exceptional sensitivity and precision.

The Master contains all reagents required for qPCR (except template, primer and labeled fluorescent probe) 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 nonspecifically annealed primers and primer-dimer formation at low temperatures during PCR setup.

The mix contains UNG (Uracil-N-Glycosylase) and dUTP instead of dTTP to eliminate carry-over contaminations of DNA from previous PCR reactions. The UNG treatment at the onset of thermal cycling removes uracil residues from dU-containing DNA and prevents it from serving as template.

The reaction chemistry of the kit is optimized for block-based PCR instruments that are compatible with the evaluation of the ROX reference signal.

ROX reference dye

The qPCR Master contains ROX passive reference dye. The dye does not take part in the PCR reaction but allows to normalize for non-PCR related signal variation and provides a baseline in multiplex reactions.

Dual-labeled DNA probes

Real-time PCR technology based on dual-labeled DNA probes provides a high sensitive and high specific PCR system with multiplexing capability. It requires two standard PCR primers and the DNA probe that hybridizes to an internal part of the amplicon. The sequence of the dual-labeled DNA probe should avoid secondary structure and primer-dimer formation.

2x qPCR ProbesMaster with UNG / ROX (red cap)

qPCR Pol, dATP, dCTP, dGTP, dUTP, reaction buffer with KCl, (NH₄)₂SO₄ and MgCl₂, UNG, ROX, stabilizers

PCR-grade water (white cap)

Preparation of the qPCR master mix

The preparation of a master mix is crucial in quantitative PCR reactions to reduce pipetting errors. Prepare a master mix of all components except template as specified. A reaction volume of 20-50 µl is recommended for most real-time instruments. Prepare 13 volumes of master mix for 12 samples or a triple-set of 4 samples. Pipet with sterile filter tips and minimize the exposure of the master mix, reaction buffer and labeled DNA probe to light. Perform the setup in an area separate from DNA preparation or analysis. No-template controls should be included in all amplifications.

| Component | Volume for 1x 50 µl master mix | Volume for 13x 50 µl master mix | Final conc. |
|--|--------------------------------|---------------------------------|-------------|
| 5x qPCR Master with UNG and ROX (red cap) | 25 µl | 325 µl | 1x conc. |
| Primer forward (10 µM) ¹⁾ | 1.5 µl | 19.5 µl | 300 nM |
| Primer reverse (10 µM) ¹⁾ | 1.5 µl | 19.5 µl | 300 nM |
| dual-labeled DNA probe (10 µM) ²⁾ | 1 µl | 13 µl | 200 nM |
| PCR-grade water | Fill up to 40 µl | Fill up to 520 µl | |

- 1) The optimal concentration of each primer may vary from 100 to 500 nM.
- 2) Optimal results may require a titration of DNA probe concentration between 50 and 800 nM.

Dispensing the master mix

Vortex the master mix thoroughly to assure homogeneity. Dispense 40 µl to a PCR tube or each well of the PCR plate.

Addition of template DNA

Add 10 µl of sample template DNA to each reaction vessel containing 40 µl master mix and cap or seal the tube / plate. Do not exceed 500 ng DNA per 50 µl reaction as final concentration. Tubes or plates should be centrifuged before cycling to remove possible bubbles.

Recommended cycling conditions

| | | | |
|--|------------------------|----------------------|--------|
| UNG treatment ³⁾ | 50 °C | 2 min | 1x |
| Initial denaturation and polymerase activation | 95 °C | 2 min | 1x |
| Denaturation | 95 °C | 15 sec | 40-50x |
| Annealing and elongation | 60-65 °C ⁴⁾ | 30 sec ⁵⁾ | |

- 3) Cycling step 1 is only required if an UNG (Uracil-N-Glycosylase) treatment is applied.
- 4) The annealing temperature depends on the melting temperature of the primers and DNA probe used.
- 5) The elongation time depends on the length of the amplicon. A time of 30 sec for a fragment of up to 500 bp is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters, especially of the annealing temperature may be necessary for each new combination of template DNA, primer pair, and DNA probe.