

qPCR ProbesMaster

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

Real-Time PCR

Cat.-No.	Amount
PCR-311S	100 reactions x 50 µl
PCR-311L	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

Description

qPCR ProbesMaster 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 dUTP instead of dTTP and allows an UNG (Uracil-N-Glycosylase) treatment at the onset of thermal cycling to prevent carry-over contaminations of DNA from previous PCR reactions. The reaction chemistry of the mix is optimized for block-based PCR instruments. The mix can also be used with ROX reference dye in PCR instruments that are compatible with the evaluation of the ROX signal. In this case, the ROX dye should be added as 1x concentration to the PCR reaction.

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 (red cap)

qPCR Pol, dATP, dCTP, dGTP, dUTP, reaction buffer with KCl, (NH₄)₂SO₄ and MgCl₂, 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 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 (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
UNG (PCR-353) ³⁾	0.2 µl	2.6 µl	4x10 ⁻³ u/µl
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.
- 3) Only required if an UNG (Uracil-N-Glycosylase) treatment to prevent carry-over contaminations of DNA should be applied. UNG is not provided by this kit.

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 ⁴⁾	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 ⁵⁾	30 sec ⁶⁾	

- 4) Cycling step 1 is only required if an UNG (Uracil-N-Glycosylase) treatment is applied.
- 5) The annealing temperature depends on the melting temperature of the primers and DNA probe used.
- 6) 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.