

Data Sheet (22.02.2010)

mi-real-time Probe Master (ROX+)

2 x ready-to-use hot start mix for quantitative real-time PCR using labeled DNA probes and ROX reference dye

Cat.-No.	Amount
mi-E2006S	100 rx (50 µl)
mi-E2006L	500 rx (50 µl)

Only for *in vitro* use!
For research only!

2x 1.25 ml/ 10x 1.25 ml
for 100/ 500 rx of 50µl final volume

2x mi-real-time Probe Master (ROX+) (blue cap)

- Taq Polymerase: 0.05 u/ µl
- dNTPs (dATP, dCTP, dGTP, dUTP) (200 µM)
- reaction buffer with KCl and MgCl₂ (3 mM)
- ROX stabilizer

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.

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

Storage

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

Storage at 4 °C for up to 3 months possible.

mi-real-time Probe Master (ROX+) must be stored in the dark.

Description

mi-real-time Probe Master (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 Labeled Fluorescent Probes, e.g. for 5'Nuclease Assays or Hybridization 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.

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. 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	Final conc.
2x real-time Probe Master (ROX+) (blue cap)	25 µl	1x conc.
Primer forward (10 µM) ¹⁾	1.5 µl	300 nM
Primer reverse (10 µM) ¹⁾	1.5 µl	300 nM
dual-labeled DNA probe (10 µM) ²⁾	1 µl	200 nM
PCR- grade water	Fill up to 45 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.

If a treatment with UNG (Uracil-N-Glycosylase) is necessary, add 0.2 units of UNG to the master mix (final conc. 4×10^{-3} u/ µl). UNG is not provided in this kit.

Dispensing the master mix

Vortex the master mix thoroughly, to assure homogeneity. Dispense 45 µl to a PCR tube or each well of the PCR plate.

Addition of template DNA

Add 5 µl of sample template DNA to each reaction vessel containing 45 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

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

³⁾ The annealing temperature depends on the melting temperature of the primers and DNA probe used.

⁴⁾ The elongation time depends on the length of the amplicon. A time of 1 min for a fragment of up to 500 bp is recommended.

If an UNG (Uracil-N-Glycosylase) treatment is applied, start with one cycling step at 50 °C for 2 min.

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.