

qPCR Core Kit with ROX

Kit for quantitative real-time PCR using labeled DNA probes

Real-Time PCR

Cat.-No.	Amount
PCR-332S	100 reactions x 50 µl
PCR-332L	500 reactions x 50 µl

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20°C, avoid frequent thawing and freezing
qPCR Buffer +ROX must be stored in the dark

Description

qPCR Core Kit with ROX is designed for the quantitative real-time analysis of DNA samples using DNA probe based detection. The kit is recommended for use with Dual-Labeled Fluorescent Probes, e.g. TaqMan®, Molecular Beacons or FRET probes. It provides a 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 high specificity and sensitivity of the Core Kit 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 included dNTP 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 kit is optimized for block-based PCR instruments that are compatible with the evaluation of the ROX reference signal.

ROX reference dye

The 10x qPCR reaction buffer +ROX 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.

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

qPCR Pol (red cap)

5 units/µl

dNTP Mix incl. dUTP (white cap)

10 mM dATP, 10 mM dCTP, 10 mM dGTP,
20 mM dUTP

10x qPCR Buffer +ROX complete (green cap)

200 mM Tris-HCl (pH 8.5), KCl, (NH₄)₂SO₄, stabilizers,
5 µM ROX passive reference, 30 mM MgCl₂

10x qPCR Buffer +ROX without MgCl₂ (blue cap)

200 mM Tris-HCl (pH 8.5), KCl, (NH₄)₂SO₄, stabilizers,
5 µM ROX passive reference

MgCl₂ Stock Solution (yellow cap)

25 mM MgCl₂

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.
10x qPCR Buffer complete (green cap)	5 μl	65 μl	1x conc.
dNTP Mix incl. dUTP (white cap)	1 μl	13 μl	200 μM
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) ²⁾	0.2 units	2.6 units	4×10^{-3} u/ μl
qPCR Pol (red cap)	0.25 μl	3.25 μl	0.025 u/ μl
PCR-grade water	Fill up to 45 μl	Fill up to 585 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) UNG (Uracil-N-Glycosylase) can be used to prevent carry-over contaminations of DNA from previous PCR reactions. UNG is not provided by this kit.

Optimization of MgCl₂ concentration

A concentration of 3.0 mM Mg²⁺ as provided by the 10x qPCR Buffer +ROX complete (green cap) is recommended for most applications. For an individual optimization use the reaction buffer without MgCl₂ and add MgCl₂ Stock Solution as shown in the table below.

per 50 μl PCR reaction				
MgCl ₂ Stock Sol.	4 μl	6 μl	8 μl	12 μl
Final MgCl ₂ conc.	2 mM	3 mM	4 mM	6 mM

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

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 MgCl₂ concentration and the annealing temperature may be necessary for each new combination of template DNA, primer pair, and DNA probe.