

qPCR GreenMaster with ROX

Master mix for real-time qPCR with green-fluorescent DNA stain

Real-Time PCR

Cat.-No.	Amount
PCR-314S	100 reactions x 50 µl
PCR-314L	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 GreenMaster with ROX must be stored in the dark

Description

qPCR GreenMaster with ROX is designed for the quantitative real-time analysis of DNA samples using the fluorescent DNA stain EvaGreen®. The fluorescent dye in the master mix intercalates into the amplification product during the PCR process and enables the rapid analysis of target DNA without the need to synthesize sequence-specific labeled 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 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 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 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.

EvaGreen® Fluorescent DNA Stain

EvaGreen® Fluorescent DNA Stain is a superior DNA intercalator dye specially developed for DNA analysis applications including real-time PCR (qPCR) and high-resolution DNA melting curve analysis (HRM). Upon binding to DNA, the non-fluorescent dye becomes highly fluorescent while showing no detectable inhibition to the PCR process. The dye is extremely stable both thermally and hydrolytically, providing convenience during routine handling.

The high quantum yield, excellent stability and lowest

qPCR GreenMaster with ROX (red cap)

2x conc.

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

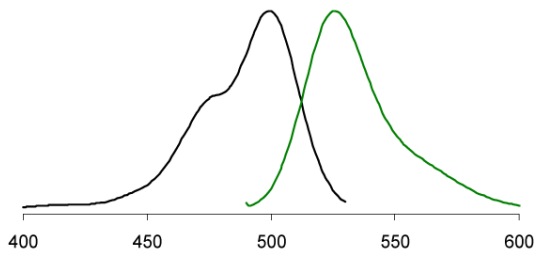
PCR-grade water (white cap)

inhibition toward PCR makes it the ideal fluorophore in real-time PCR applications and a superior replacement for the widely used SYBR® Green I dye.

Spectroscopic data

Excitation maximum: $\lambda_{Ex} = 500 \text{ nm}$ (bound to DNA)

Emission maximum: $\lambda_{Em} = 530 \text{ nm}$ (bound to DNA)



Excitation (left) and emission (right) spectra of EvaGreen® bound to dsDNA in PBS buffer (pH 7.3).

To perform the EvaGreen-based assay simply select the optical setting for SYBR® Green or FAM on the detection instrument.

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 with UNG (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
UNG (PCR-353) ²⁾	0.2 μl	2.6 μl	$4 \times 10^{-3} \text{ u}/\mu\text{l}$
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) 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 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 should be 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.

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