



qPCR GreenMaster highROX

Master mix for real-time qPCR with EvaGreen® fluorescent DNA stain

Cat. No.	Amount
PCR-368S	2 x 1,25 ml (2x conc.)
PCR-368L	10 x 1,25 ml (2x conc.)

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles, store dark
Storage at 4 °C for up to 3 months possible.

Shelf Life: 12 months

Form: liquid

Concentration: 2x conc.

Spectroscopic Properties: λ_{exc} 500 nm (bound to DNA), λ_{em} 530 nm (bound to DNA)

Description:

qPCR GreenMaster highROX is designed for quantitative real-time analysis of DNA samples including High Resolution Melting (HRM) curve analysis. The mix contains all reagents required for qPCR (except template and primers) in a premixed 2x concentrated ready-to-use solution. It is recommended for routine PCR applications, high throughput PCR or genotyping and provides an improved specificity and sensitivity when amplifying low-copy-number targets or working with complex backgrounds.

The mix is based on an optimized hot-start polymerase. Its activity is blocked by antibody 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 fluorescent DNA stain EvaGreen® intercalates into the amplification product during the PCR process and allows the direct quantification of target DNA without the need to synthesize sequence-specific labeled probes (i.g. TaqMan® Probes).

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

High Resolution Melting (HRM) analysis is a powerful tool for detection of mutations, polymorphisms and epigenetic differences in DNA samples. It is a fast and cost effective alternative to other genotyping technologies.

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.

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

ROX reference dye:

The qPCR GreenMaster with highROX contains 500 nM ROX passive reference dye in the final assay. 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.

Content:

qPCR GreenMaster highROX (red cap)

antibody-blocked hot start polymerase, dATP, dCTP, dGTP, dTTP, KCl, $(NH_4)_2SO_4$, $MgCl_2$, EvaGreen® DNA intercalator dye, 1 μ M ROX, additives and stabilizers

PCR-grade water



qPCR GreenMaster highROX

Master mix for real-time qPCR with EvaGreen® fluorescent DNA stain

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	20 µl assay	50 µl assay	final conc.
qPCR Green-Master with highROX	10 µl	25 µl	1x
primer forward (10 µM) ¹⁾	0.6 µl	1.5 µl	300 nM
primer reverse (10 µM) ¹⁾	0.6 µl	1.5 µl	300 nM
template DNA	x µl	x µl	<500 ng/assay
PCR-grade water	fill up to 20 µl	fill up to 50 µl	-

¹⁾ The optimal concentration of each primer may vary from 100 to 500 nM.

Dispensing the master mix:

Vortex the master mix thoroughly to assure homogeneity and dispense the mix into real-time PCR tubes or wells of the PCR plate.

Addition of template DNA:

Add the remaining x µl of sample/template DNA to each reaction vessel containing the master mix and cap or seal the tubes/plate. Do not exceed 500 ng DNA per 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	35-45x
Annealing and elongation	60-65 °C ³⁾	1 min ⁴⁾	35-45x

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.

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.

EvaGreen® is a registered trademark and licensed for sale by Biotium, Inc., Hayward, CA, USA

SYBR® is a registered trademark of Invitrogen Corporation, Carlsbad, California, USA

Related Products:

Dual-labeled DNA probes
Custom primers