DATA SHEET

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qPCR SybrMaster + ROX

2x Master mix for real-time qPCR with SYBR® Green fluorescent DNA stain and ROX reference dye

Cat. Nº.	Amount
☐ PCK-107XS	1 x 1,25 mL (XS) - 50 reactions of 50 µL
☐ PCK-107S	2 x 1,25 mL (XS) - 100 reactions of 50 µL
☐ PCK-107M	4 x 1,25 mL (XS) - 200 reactions of 50 µL
☐ PCK-107L	10 x 1,25 mL (XS) - 500 reactions of 50 µL
☐ PCK-107XL	20 x 1,25 mL (XS) - 1.000 reactions of 50 µL
☐ PCK-107XXL	40 x 1,25 mL (XS) - 2.000 reactions of 50 µL

Concentration:
2 x conc.

Shipping:
Shipped on blue ice

Storage Conditions:
Store at -20 °C (Avoid freeze/thaw cycles, store in dark).

For *in vitro* use only!

Form:
Liquid

Shelf Life:
12 months

Spectroscopic Properties: λ_{exc} 494 nm (bound to DNA), λ_{em} 521 nm (bound to DNA)

Kit contents:

qPCR SybrMaster (ambar microtube)
Antibody-blocked hot start polymerase, dATP, dCTP, dGTP, dUTP, KCl, (NH4)2SO4, MgCl2, SYBR® Green DNA intercalator dye, additives and stabilizers

ROX reference dye (#PCK-121)
25 µM ROX Reference Dye.

SYBR® Green Fluorescent DNA Stain:
SYBR® Green Fluorescent DNA Stain is a superior DNA intercalator dye specially developed for DNA analysis applications including real-time PCR (qPCR). Upon binding to DNA, the non-fluorescent dye becomes highly fluorescent while showing no detectable inhibition to the PCR process. SYBR® Green is in contrast to EvaGreen® not recommended for high-resolution melting curve analysis (HRM). To perform the SYBR® Green-based assay simply select the optical setting for SYBR® Green on the detection instrument.

ROX 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.

Description:
qPCR SybrMaster + ROX is designed for quantitative real-time analysis of DNA samples. 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 SYBR® Green 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.e. TaqMan® Probes).

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 master mix and ROX 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 [ ]
qPCR SybrMaster	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
ROX (25 µM)[2]	0,04 or 0,4 µL	0,1 or 1 µL	50 or 500 nM
PCR-grade water	to 20 µL	to 50 µL	-

[1] The optimal concentration of each primer may vary from 100 to 500 nM.
[2] ROX reference dye concentration depends on the equipment used.

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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.

NOTE: Keep all solutions containing mastermix and ROX protected from light

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:

Step		Temp.	Time
UNG treatment[3]		50 °C	2 min
Initial denaturation and polymerase activation		95 °C	2 min
35-45 cycles	Denaturation	95 °C	15 s
	Annealing and Elongation	60-65 °C[4]	1 min[5]

[3]Cycling step 1 is only required if an UNG (Uracil-N-Glycosylase) i added to the reaction Mix. **This mix does not contain UNG.**

[4]The annealing temperature depends on the melting temperature of the primers used.

[5]The elongation time depends on the length of the fragments to be amplified. 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 and primer pair.

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