DATA SHEET

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qPCR GreenMaster lowROX with UNG

2X Master mix for real-time qPCR
Green-fluorescent DNA Stain

Cat. Nº.	Amount
☐ PCK-104XS	1 x 1,25 mL (XS) - 50 reactions of 50 µL
☐ PCK-104S	2 x 1,25 mL (XS) - 100 reactions of 50 µL
☐ PCK-104M	4 x 1,25 mL (XS) - 200 reactions of 50 µL
■ PCK-104L	10 x 1,25 mL (XS) - 500 reactions of 50 µL
☐ PCK-104XL	20 x 1,25 mL (XS) - 1.000 reactions of 50 µL
☐ PCK-104XXL	40 x 1,25 mL (XS) - 2.000 reactions of 50 µL

Concentration:
2 x conc.

Shipping:
Shipped on blue ice

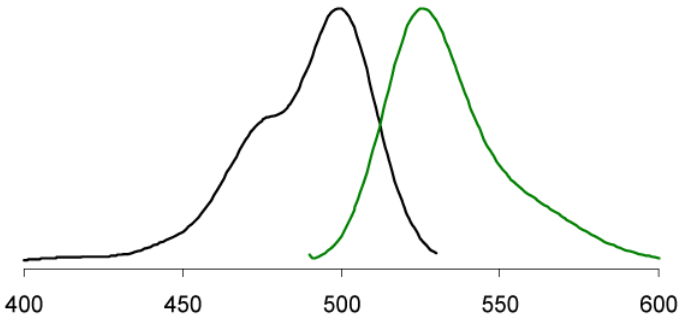
Storage Conditions:
Store at -20 °C (Avoid freeze/thaw cycles, store in dark). Store at 4 °C for up to 3 months possible.

For in vitro **use only!**

Form:
Liquid

Shelf Life:
12 months

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



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

Description:
qPCR GreenMaster low ROX with UNG 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 formations at low temperatures during PCR setup.

The mix contains UNG (Uracil-N-Glycosylase) and dUTP instead of dTTP to eliminate carry-over contamination of DNA from previous PCR reactions. The UNG treatment at the onset of thermal cycling removes uracil residues from dU-containing DNA and prevents it from serving as template. The reaction chemistry of the kit is optimized for instruments that are compatible with the evaluation of a low ROX reference signal.

Kit contents:
qPCR Green Master low ROX with UNG (red cap)
qPCR Pol, dATP, dCTP, dGTP, dUTP, UNG EvaGreen®, 100 nM ROX, reaction buffer with KCl, $(NH_4)_2SO_4$, $MgCl_2$ and stabilizers.

ROX reference dye:
The qPCR GreenMaster with lowROX contains 50 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.

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 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.

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

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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 [ ]
qPCR Green Master lowROX with UNG	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
PCR-grade water	to 20 µL	to 50 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 providet by this kit.

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:

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) treatment is 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 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, primer pair and DNA probe.