DATA SHEET

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qPCR GoldMaster

2X Master mix for real-time qPCR with EvaGreen® and GoldTaq DNA Polymerase

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

Concentration:
2 x conc.

Shipping:
Shipped on blue ice

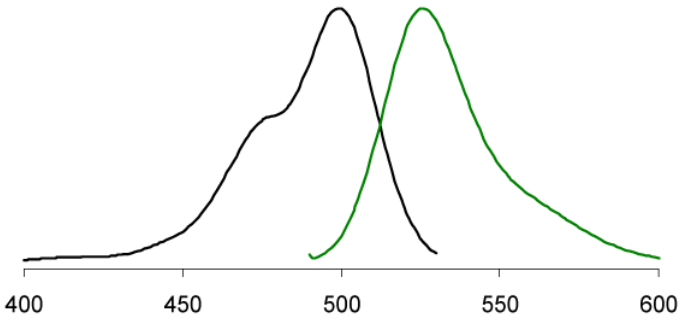
Storage Conditions:
Store at -20 °C (Avoid freeze/thaw cycles, store in dark).

For in vitro **use only!**

Form:
Liquid

Shelf Life:
12 months

Spectroscopic Properties:
λ_{exc} 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 GoldMaster is designed to provide superior specificity and sensitivity 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 and enables the rapid analysis of target DNA without 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 precision. The qPCR GoldMaster contains all reagents required for qPCR (except template and primer) in a premixed 2x concentrated ready-to-use solution. The high specificity and sensitivity is achieved by an chemically modified hot-start polymerase. Its activity is blocked at ambient temperature and switched on automatically in a hot start initial cycle allowing flexibility in the reaction set up. The thermal activation prevents the extension of nonspecifically annealed primers and primer-dimer formations.

The mix contains dUTP instead of dTTP and allows an UNG (UracilN-Glycosylase) treatment to prevent carry-over contaminations of DNA from previous PCR reactions.

The mix can also be used with ROX reference dye (#PCR-121) in PCR instruments that are compatible with the evaluation of the ROX signal. In this case, the ROX dye should be added as 1x concentration to the PCR reaction.

Kit contents:
qPCR GoldMaster (red cap)
GoldTaq DNA Polymerase, dATP, dCTP, dGTP, dUTP, EvaGreen®, reaction buffer with KCl, $(NH_4)_2SO_4$, $MgCl_2$ and stabilizers.

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.

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.

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Component	20 µL assay	50 µL assay	Final [ ]
qPCR GoldMaster	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
UNG[2]	0.2 µL	0.2 µL	0.2 U/assay
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 NOT PROVIDED IN 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	10 min
50 cycles	Denaturation	94 °C	15 sec
	Annealing	60-65 °C[4]	15 - 60 sec
	Elongation	72 °C	1 min/kb

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.