DATA SHEET

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qPCR ProbesMaster + ROX + UDG

2X Master mix for real-time PCR using labeled DNA probes with ROX and UDG

Cat. Nº.	Amount
☐ PCK-114XS	1 x 1,25 mL (XS) - 50 reactions of 50 µL
☐ PCK-114S	2 x 1,25 mL (XS) - 100 reactions of 50 µL
☐ PCK-114M	4 x 1,25 mL (XS) - 200 reactions of 50 µL
■ PCK-114L	10 x 1,25 mL (XS) - 500 reactions of 50 µL
☐ PCK-114XL	20 x 1,25 mL (XS) - 1.000 reactions of 50 µL
☐ PCK-114XXL	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

Description:
qPCR ProbesMaster + ROX + UDG is designed for the quantitative real-time analysis of DNA samples using DNA probe based detection. The master mix is recommended for use with Dual Labeled Fluorescent Probes, e.g. TaqMan®, Molecular Beacons or FRET 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, primer and labeled fluorescent probe) 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 dUTP instead of dTTP and allows an UDG (Uracil-DNA-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.

Kit contents:

qPCR ProbesMaster (#PCK-110 - red cap)
qPCR Pol, dATP, dCTP, dGTP, dUTP, reaction buffer with KCl, $(NH_4)_2SO_4$, $MgCl_2$ and stabilizers.

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

UDG (#ENZ-125)
Uracil-DNA-Glicosilase 1U/µL)

Dual-labeled DNA Probes (NOT PROVIDED)
Real-time PCR technology based on dual-labeled DNA probes provides a high sensitive and high specific PCR system with multiplexing capability. It requires two standard PCR primers and the DNA probe that hybridizes to an internal part of the amplicon. The sequence of the dual-labeled DNA probe should avoid secondary structure and primer-dimer formation.

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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 ProbesMaster (2x)	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
Dual-labeled probe (10 µM)[2]	0.4 µL	1 µL	200 nM
UDG (1U/µL)[3]	0.2 µL	0.2 µL	0.2 U/assay
ROX (25 µM)[4]	0,04 or 0,4 µL	0,1 or 1 µL	50 or 500 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)Optimal results may require a titration of DNA probe concentration between 50 and 800 nM.
3)Only required if an UDG (Uracil-DNA-Glycosylase) treatment to prevent carry-over contaminations of DNA should be applied. UNG IS PROVIDED IN THIS KIT.
4) ROX reference dye concentration depends on the equipment used.

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
UDG treatment[5]		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[6]	1 min[7]

5) Cycling step 1 is only required if an UDG (Uracil-DNA-Glycosylase) treatment is applied.

6) The annealing temperature depends on the melting temperature of the primers and DNA probe used.

7) 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.