DATA SHEET

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qPCR GoldMix Multiplex + UDG

Master mix for real-time multiplex qPCR with UDG/dUTP

Cat. Nº.	Amount
☐ PCK-216XS	1 x 1,25 mL
☐ PCK-216S	2 x 1,25 mL
☐ PCK-216M	4 x 1,25 mL
☐ PCK-216L	10 x 1,25 mL
☐ PCK-216XL	20 x 1,25 mL

Shipping:

Shipped on blue ice

Storage Conditions:

Store at -20 °C

Additional Storage Conditions:

Avoid freeze/thaw cycles.

Shelf Life:

12 months

For *in vitro* use only!

Kit contents:

2x qPCR GoldMix Multiplex 2x (red cap)

qPCR GoldMix (thermostable chemically modified hot start), dATP, dCTP, dGTP, dUTP, , KCl, $MgCl_2$ and stabilizers.

PCR grade water (white cap)

Description:

qPCR GoldMix Multiplex is designed for quantitative real-time analysis of DNA samples using Dual Labeled Fluorescent Probes, e.g. TaqMan®. The master mix is specially optimized for setting-up multiplex assays with 4 target sequences in a single tube. The system overcomes multiplex limitations of conventional qPCR probe mixes combining an above-average robustness for a multitude of known PCR inhibitors with an excellent sensitivity for amplification of lowest template amounts. The 2x concentrated master mix contains all reagents required for qPCR (except template and primer/probe sets) including a highly processive hot-start polymerase and ultra-pure dNTPs. The reaction chemistry of the mix is optimized for block-based PCR instruments. The mix can also be used with ROX reference dye (#PCK-121) in PCR instruments that are compatible with the evaluation of the ROX signal.

Dual-labeled DNA probes:

Real-time PCR technology based on dual-labeled DNA probes provides a high sensitive and high specific PCR system with multiplexing capability. For amplification of each target sequence a set of two PCR primers and one fluorescent DNA probe that hybridizes to an internal part of the amplicon are required. The sequence of the dual-labeled DNA probe should avoid secondary structure and primer-dimer formation.

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.
- No-template controls should be included in all amplifications.

Note: Consider the volumes for all components listed next steps to determine the correct amount of water required to reach your final reaction volume.

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Component	20 µL assay	50 µL assay	final conc.
2 X qPCR GoldMix	10 µL	25 µL	1x
Forward primer (10 µM)[1]	0,6 µL	1,5 µL	300 nM
Reverse primer (10 µM)[1]	0,6 µL	1,5 µL	300 nM
Each dual-labeled probe (10	0,4 µL	1 µL	200 nM
DNA template	x µL	x µL	<500 ng/assay
UDG (1 U/µL)	0,2 µL	0,2 µL	0,2 U/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.
2) Optimal results may require a titration of DNA probe concentration between 50 and 800 nM.

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		50 °C	2 min
Initial denaturation		95 °C	10 min
35 cycles	Denaturation	95 °C	20 s
	Annealing[1] and extension	60 °C	50 s

1)The optimal annealing temperature (AT) can be calculated for each primers as following: AT - Tm - 5 °C with Tm - 2 °C x (A + T) + 4 °C x (G + C).

Please note that primers should be designed to show minimal differences in there melting temperatures (Tm).

For optimal specificity and amplification an individual optimization of the recommended parameters, especially of the annealing temperature and different step at 72 °C for extension may be necessary for each new combination of template DNA, primer pair and DNA probe.