

DATA SHEET

**qPCR GoldMix Multiplex**

Master mix for real-time multiplex qPCR

Cat. Nº.	Amount
<input type="checkbox"/> PCK-215XS	1 x 1,25 mL
<input type="checkbox"/> PCK-215S	2 x 1,25 mL
<input type="checkbox"/> PCK-215M	4 x 1,25 mL
<input type="checkbox"/> PCK-215L	10 x 1,25 mL
<input type="checkbox"/> PCK-215XL	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, MgCl₂ 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.
- Is recommended to perform the setup in a separate area from the analysis.
- 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 μ L assay	50 μ L assay	final conc.
qPCR GoldMix Multiplex	10 μ L	25 μ L	1x
Forward primer (10 μ M) ¹	0,6 μ L	1,5 μ L	300 nM
Reverse primer (10 μ M) ¹	0,6 μ L	1,5 μ L	300 nM
Each dual-labeled probe (10 μ M) ²	0,4 μ L	1 μ L	200 nM
DNA template	x μ L	x μ L	<500 ng/assay
PCR grade water	fill up to 20 μ L	fill up to 50 μ L	-

- 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 μ L 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
Initial denaturation	95 °C	10 min
35 cycles	Denaturation	95 °C 20 s
	Annealing and extension	60 °C 50 s

- 1) The optimal annealing temperature (AT) can be calculated for each primers as following: $AT = T_m - 5\text{ °C}$ with $T_m = 2\text{ °C} \times (A + T) + 4\text{ °C} \times (G + C)$. Please note that primers should be designed to show minimal differences in there melting temperatures (T_m).

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.