

DATA SHEET



qPCR SybrMaster Lyophilisate

Lyophilised real-time PCR Master Mix with SYBR® Green fluorescent DNA stain

Cat. Nº.	Amount
<input type="checkbox"/> PCK-131S	192 reactions of 20 µL
<input type="checkbox"/> PCK-131L	960 reactions of 20 µL

Shipping:

shipped at ambient temperature

Storage Conditions:

store at ambient temperature

Additional Storage Conditions:

Store in an aluminium-coated bag or on a dry place.

Lyophilisates may hydrate at humidity levels >70 % when sealing is opened.

For in vitro use only!

Shelf Life:

12 months

Description:

qPCR SybrMaster Lyophilisate is designed for quantitative real time analysis of DNA samples using the fluorescent DNA stain SYBR® Green. 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 lyophilisate contains all reagents required for qPCR (except template, primer and labeled fluorescent probe) in a single bead. The high specificity and sensitivity of the mix based on 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 formation at low temperatures during PCR setup.

Content:

qPCR ProbesMaster Lyophilisate

antibody-blocked hot start polymerase, dATP, dCTP, dGTP, dTTP, KCl, (NH₄)₂SO₄, MgCl₂, SYBR® Green DNA intercalator dye, additives and stabilizers

PCR-grade water

Handling

qPCR Master Lyophilisate is delivered in PCR reaction tube strips or 96-well plates preloaded with a complete qPCR master mix in a dry, room temperature stable format. The lyophilisate combines highest performance with convenience of use and stability. There is no need for freezing, thawing or pipetting on ice. The few remaining pipetting steps minimize the risk of errors or contaminations. Each vial contains all components (except primers and template) required for a 20 µl real-time PCR assay. To perform PCR, only fill up the vials with a primer mix and add DNA template. The lyophilisate can also be used with ROX reference dye in PCR instruments that are compatible with the evaluation of the ROX signal. In this case, the ROX dye (#PCR-351) should be added as 1x concentration to the PCR reaction.

Recommended PCR assay:

Component	stock conc.	final conc.	Vol. for 20µL mix
Primer forward ¹	10 µM	300 nM	0,6 µL
Primer reverse ¹	10 µM	300 nM	0,6 µL
PCR-grade water	-	-	Fill up to 15 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.

Preparation of the primer mix

Prepare 13 volumes of primer mix for 12 samples or a triple-set of 4 samples as specified. 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 (NTC) should be included in all applications.

Dispensing the master mix

Vortex the primer mix thoroughly to assure homogeneity. Dispense 15 µl to each PCR tube or well of the plate.

Addition of template DNA

Add 5 µl of template DNA (or no-template controls) to each reaction vessel and cap or seal the tube / plate. Do not exceed 200 ng DNA per reaction as final concentration. Tubes or plates should be centrifuged before cycling to remove possible bubbles.

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Recommended cycling conditions:

Step		Temp.	Time
Initial denaturation and polymerase activation		95 °C	2 min
30 - 40 cycles	Denaturation	95 °C	15 s
	Annealing and	55-65 °C ³	20 s ⁴
	Elongation	72 °C ³	

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

6) The elongation time depends on the length of the amplicon. A time of 30 s 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 and primer pair.

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