DATA SHEET

Jena Bioscience

qPCR ProbesMaster Lyophilisate

lyophilised real-time PCR Master Mix for dual labeled probes

Cat. No.	Amount
PCR-156S	192 reactions x 20 µl
PCR-156L	960 reactions x 20 µl

For *in vitro* use only!

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.

Shelf Life: 12 months in sealed package

Description:
qPCR ProbesMaster Lyophilisate is designed for quantitative real-time analysis of DNA samples using DNA probe based detection. The lyophilisate 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 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, $(NH_4)_2SO_4$, $MgCl_2$, 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, Dual Labeled Probes and template) required for a 20 µl real-time PCR assay.
To perform PCR, only fill up the vials with a primer/probe premix 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.

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. 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.

Preparation of the primer/probe mix
The preparation of a primer/probe premix is recommended in quantitative PCR reactions to reduce pipetting errors. 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

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or analysis. No-template controls (NTC) should be included in all amplifications.

Recommended PCR assay:

Comp.	stock conc.	final conc.	Volume for 1x 20 µl mix
forward Primer[1)]	10 µM	300 nM	0.6 µl
reverse Primer[1)]	10 µM	300 nM	0.6 µl
Dual-Labeled DNA probe[2)]	10 µM	200 nM	0.4 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.

Dispensing the master mix
Vortex the primer/probe 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 10 ng DNA per reaction as final concentration. Tubes or plates should be centrifuged before cycling to remove possible bubbles.

Recommended cycling conditions:

Initial denaturation and polymerase activation	95 °C	2 min	1x
Denaturation	95 °C	15 sec	40-50x
Annealing and Elongation	60-65 °C[3)]	1 min[4)]	40-50x

[3)] The annealing temperature depends on the melting temperature of the primers and DNA probe used.
[4)] The elongation time depends on the length of the amplicon. 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, primer pair and DNA probe.