DATA SHEET

Jena Bioscience

Liquid qPCR ProbesMaster for Lyophilization

Liquid real-time PCR master mix for lyophilization of qPCR assays
2.5 x conc. master mix

Cat. No.	Amount
PCR-188-1ML	1 ml
PCR-188-10ML	10 ml
PCR-188-100ML	100 ml

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C, avoid freeze/thaw cycles

Shelf Life: 6 months

Form: liquid

Concentration: 2.5x conc.

Description:
Liquid qPCR ProbesMaster for Lyophilization is a liquid 2.5 x conc. master mix designed for custom-specific production of freeze-dried real-time PCR assays. Its chemistry is optimized for using DNA probe based detection with Dual Labeled Fluorescent Probes, e.g. TaqMan®, Molecular Beacons or FRET probes.
The master mix contains all reagents required for qPCR (except template, primer and labeled fluorescent probe) in a well balanced composition. The high specificity and sensitivity of the mix is based on a hot-start polymerase with blocked activity at ambient temperature.
The mix can also be used in combination with ROX reference dye (#PCR-356) in PCR instruments that are compatible with the evaluation of the ROX signal.

Content:
Liquid qPCR ProbesMaster for Lyophilization
2.5 x conc. master mix containing antibody-blocked hot start polymerase, nucleotides, reaction buffer, additives and stabilizers

Preparation of the 2 x conc. master mix
Liquid qPCR ProbesMaster for Lyophilization comes as 2.5 x conc. master mix and must be diluted to 2.0 x concentration prior to lyophilization. Lyophilization of 10 µl, 2 x conc. master mix per tube or well to obtain a final assay volume of 20 µl is recommended. Designing of multiplex reactions with up to 4 primer-probe sets is possible but may require an additional effort for assay optimization.

Recommended concentrations of components:

Comp.	final conc.
Liquid qPCR ProbesMaster for Lyophilization	2 x conc.
forward Primer 1 [1)]	300 nM
reverse Primer 1 [1)]	300 nM
Dual-Labeled probe 1 [2)]	200 nM
forward Primer 2 [1)]	300 nM
reverse Primer 2 [1)]	300 nM
Dual-Labeled probe 2 [2)]	200 nM
ROX Reference Dye (PCR-356) [3)]	500 nM
PCR-grade Water (PCR-258)	fill up to 2 x conc.

[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)] Optional if ROX Refernce Dye is required for the assay design and compatible with the used cycler. 500 nM is recommended for high ROX assays.

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Dispensing the master mix
Vortex the 2 x conc. mix prepared above thoroughly to assure homogeneity. Dispense 10 µl to each PCR tube or well of the plate.

Lyophilization of the master mix
Use a freeze dryer or sublimator for freeze drying the prepared master mix. Follow the instructions provided by the freeze-dryer manufacturer.

Addition of template DNA
To obtain a final assay volume of 20 µl, rehydrate the lyophilisate cakes in 20 µl template DNA / purified DNA or 20 µl PCR-grade Water for no-template controls 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 bubbles.

Recommended cycling conditions:

Initial denaturation and polymerase activation	95 °C	2 min	1 x
Denaturation	95 °C	15 sec	35-45 x
Annealing and Elongation	60-65 °C [4)]	30 sec [5)]	35-45 x

[4)] The annealing temperature depends on the melting temperature of the primers and DNA probe used.
[5)] The elongation time depends on the length of the amplicon. A time of 30 sec for a fragment length of up to 250 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 pairs and DNA probe.