DATA SHEET

Jena Bioscience

Multiplex PCR Master Lyophilisate

lyophilised Master Mix for multiplex PCR application

Cat. No.	Amount
PCR-161S	192 reactions x 20 µl
PCR-161L	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

Description:
Multiplex PCR Master Lyophilisate is delivered in PCR reaction tube strips or 96-well plates preloaded with a complete 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.
To perform PCR, fill up the vials with primers and PCRgrade water and add DNA template. If necessary, centrifuge to remove bubbles, vortex the vials to assure homogeneity and start cycling.
The lyophilisate is specially designed for the set-up of multiplex PCR reactions. It contains an optimized composition of polymerase, nucleotides, $MgCl_2$ and stabilizing components in a specifically developed buffer system allowing the parallel amplification of a multitude of fragments in a single PCR assay.
The lyophilisate is recommended for use in routine PCR reactions and highly suitable for multiple target gene amplification in a single tube.
The high specificity and sensitivity of the mix is achieved by an optimized hot-start polymerase. Its activity is blocked at ambient temperature preventing the extension of nonspecifically annealed primers and primer-dimer formations at low temperatures during PCR setup.

Content:
Multiplex PCR Master Lyophilisate
Preloaded lyophilisates of Multiplex PCR Mastermix containing Hot Start Taq polymerase, nucleotides, optimized reaction buffer, stabilizers

PCR grade water(white cap)

Recommended PCR assay:
Prepare a primer mix to reduce pipetting errors. Pipet with sterile filter tips and perform the setup in an area separate from DNA preparation or analysis. Notemplate controls should be included in all amplifications.

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Comp.	stock conc.	final conc.	20 µl assay
Multiplex PCR Master Lyophilisate		1x	1 tube
forward Primer 1	10 µM	400 nM	0.8 µl
reverse Primer 1	10 µM	400 nM	0.8 µl
forward Primer 2	10 µM	400 nM	0.8 µl
reverse Primer 2	10 µM	400 nM	0.8 µl
forward Primer ...	10 µM	400 nM	0.8 µl
reverse Primer ...	10 µM	400 nM	0.8 µl
Template - animal genomic DNA - bacterial genomic DNA - plasmid and lambda DNA			10-200 ng 1-50 ng 1-5 ng
PCR-grade water			fill up to 20 µl

Recommended cycling conditions:

Initial denaturation	95 °C	10 min	1x
Denaturation	95 °C	30 sec	30-50x[2)]
Annealing[1)]	58-64 °C	40 sec	30-50x[2)]
Elongation[3)]	72 °C	1 min/kb	30-50x[2)]
Final elongation	72 °C[5)]	5 min	1x

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[1] The optimal annealing temperature (AT) can be calculated for each primer as following:
AT = T_m - 5°C with T_m = 2°C*(A+T) + 4°C*(G+C)
Please note that primers should be designed to show minimal differences in there melting temperatures (T_m).
[2] Cycle numbers are recommended as following:
- animal genomic DNA
10-50 ng: 35-50 cycles
50-200 ng: 30-45 cycles
- bacterial genomic DNA
1-5 ng: 35-50 cycles
5-50 ng: 30-40 cycles
- plasmid and lambda DNA
1-5 ng: 30-40 cycles
[3] The elongation time depends on the length of the fragments to be amplified. A time of 1 min per kb is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary for each new primer-template combination.