DATA SHEET

Jena Bioscience

Multiplex PCR Master

Master mix for multiplex PCR

Cat. No.	Amount
PCR-110S	2 x 1,25 ml (2x conc.)
PCR-110L	10 x 1,25 ml (2x conc.)

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid

Concentration: 2x conc.

Description:
Multiplex PCR Master 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 master mix contains all reagents (except primer and template) in a 2x concentrated ready-to-use solution.
The kit 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 a chemically inhibited 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:
2x Multiplex PCR Master (red cap)
master mix containing Hot Start Taq polymerase, nucleotides, optimized reaction buffer and stabilizers

PCR grade water (white cap)

Recommended 50 µl PCR assay:
Prepare a master mix of all components except template to reduce pipetting errors. A reaction volume of 20-50 µl per assay is recommended for most PCR cyclers. Pipet with sterile filter tips and perform the setup in an area separate from DNA preparation or analysis. No-template controls should be included in all amplifications.

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component	stock conc.	final conc.	50 µl assay
Multiplex PCR Master	2x	1x	25 µl
forward primer 1	10 µM	400 nM	2 µl
reverse primer 1	10 µM	400 nM	2 µl
forward primer 2	10 µM	400 nM	2 µl
reverse primer 2	10 µM	400 nM	2 µl
forward primer ...	10 µM	400 nM	2 µl
reverse primer ...	10 µM	400 nM	2 µl
Template a) animal genomic DNA b) bacterial genomic DNA c) plasmid and lambda DNA	-	-	a) 10-200 ng b) 1 - 50 ng c) 1 - 5 ng
PCR-grade water	-	-	fill up to 50 µl

Recommended cycling conditions:

Initial denaturation	95 °C	12 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 min	1x

[1)]The optimal annealing temperature (AT) can be calculated for each primer as following:
AT = T_m - 5 °C with T_m = 2 °C x (A+T) + 4 °C x (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/kb is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary for each new template DNA and/or primer pair.

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