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 general laboratory use.

Shipping: shipped on gel packs

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.

Multiplex PCR Master

Master mix for multiplex PCR

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.