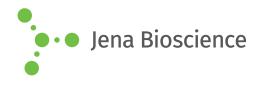
# **DATA SHEET**





## **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)

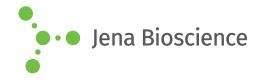
master mix containing Hot Start Taq polymerase, nucleotides, optimized reaction buffer and stabilizers

### 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  $\mu$ l 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.

# **DATA SHEET**





# **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 μΜ	400 nM	2 μl
reverse primer 1	10 μΜ	400 nM	2 μl
forward primer 2	10 μΜ	400 nM	2 μl
reverse primer 2	10 μΜ	400 nM	2 μl
forward primer	10 μΜ	400 nM	2 μl
reverse primer	10 μΜ	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

2)Cycle numbers are recommended as following:

animal genomic DNA
 50 mg 25 50 mg

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

<sup>3)</sup>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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## Recommended cycling conditions:

Initial	95 °C	12 min	1x
denaturation			
Denaturation	95 °C	30 sec	30 - 50x <sup>2)</sup>
Annealing <sup>1)</sup>	58 - 64 °C	40 sec	30 - 50x <sup>2)</sup>
Elongation <sup>3)</sup>	72 °C	1 min/kb	30 - 50x <sup>2)</sup>
Final elongation	72 °C	5 min	1x
Elongalion	I		

<sup>1)</sup>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  $(T_{\rm m})$ .