DATA SHEET

Multiplex PCR Master Mix

Master mix for multiplex PCR

Cat. Nº.	Amount
■ POL-140XS	50 reactions
□ POL-140S	100 reactions
□ POL-140M	200 reactions
□ POL-140L	500 reactions
□ POL-140XL	1.000 reactions

Shipping:
Shipped on blue ice

Storage Conditions:
Store at -20 °C

Additional Storage Conditions:
Avoid freeze/thaw cycles.

Shelf Life:
12 months

For *in vitro* use only!

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 clinical 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.

Kit contents:
2x Multiplex PCR Master (purple cap)
Master mix of thermostable Hot Start DNA polymerase, dATP, dCTP, dGTP, dTTP, KCl, $MgCl_2$ and stabilizers.

PCR grade water (white cap)

PCR Reaction Setup

A reaction volume of 10-50 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. Thaw, mix, and briefly centrifuge each component before use. Add the following components to a microcentrifuge tube:

1. Prepare PCR master mix

Note: Consider the volumes for all components listed next steps to determine the correct amount of water required to reach your final reaction volume.

Components	50 µL rxn	[final]
Water, grade PCR	To 50 µl	
2 X Multiplex PCR Master	25 µl	1X
Forward primer 1 (10 µM)	0,5 - 2,5 µl	0,1 – 0,5 µM
Reverse primer 1 (10 µM)	0,5 - 2,5 µl	0,1 – 0,5 µM
DNA template	x	10 pg – 1 µg**

**genomic DNA: 1 ng-1µg; plasmidial ou viral DNA: 1 pg-1 ng

Cap each tube, mix, and briefly centrifuge the content.

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3. Incubate reactions in a thermal cycler.

Recommended cycling conditions:

Step		Temp.	Time
Initial denaturation		95 °C	10 min
30-50 cycles	Denaturation	95 °C	15 - 30 sec
	Annealing [1]	58-64 °C	15 - 40 sec
	Elongation [2]	72 °C	1 min/kb
Final extension		72 °C	5 min
Hold		4 - 8 °C	

1)The optimal annealing temperature (AT) can be calculated for each primer as following: AT = Tm - 5 °C with Tm = 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 (Tm).

2)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.