DATA SHEET

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Biotecnologia ao seu alcance

Taq Pol - buffer (-) Mg^{+2}

Thermostable DNA polymerase
Thermus aquaticus, recombinant, *E. coli*

Cat. Nº.	Amount
☐ POL-114XS	250 units
☐ POL-114S	500 units
☐ POL-114M	1.000 units
☐ POL-114L	2 x 1.000 units (M)
☐ POL-114XL	4 x 1.000 units (M)
■ POL-114XXL	5 x 1.000 units (M)

Unit Definition:
One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmoles of dNTPs into an acid-insoluble form in 30 minutes at 70 °C.

Concentration:
5 units/µL

Shipping:
Shipped on blue ice

Storage Conditions:
Store at -20 °C

For *in vitro* use only!

Additional Storage Conditions:
Avoid freeze/thaw cycles

Shelf Life:
12 months

Description:
Taq Pol is recommended for use in routine PCR reactions. It is optimized for high specificity and guarantees minimal by-product formation. The buffer system is recommended for plate based PCR and automated pipetting.

The enzyme replicates DNA at 72 °C. It catalyzes the polymerization of nucleotides into duplex DNA in 5' → 3' direction in the presence of magnesium. It also possesses a 5' → 3' polymerization-dependent exonuclease replacement activity but lacks a 3' → 5' exonuclease activity.

Kit contents:
Taq DNA Pol (blue cap) - POL - 100
5 units/µl Taq DNA Polymerase in Tris-HCl pH 8.0 (25 °C), KCl, EDTA, DTT, 0.5 %, 50% (v/v) Glycerol and stabilizers.

Taq Reaction Buffer (-) Mg^{+2} (red cap) - 10x conc.
Tris-HCl pH 8.5 (25°C), KCl.

$MgCl_2$ Stock Solution (yellow cap)
50 mM $MgCl_2$

PCR Reaction Setup

The PCR procedure below shows appropriate volumes for a single 50 µL reaction. For multiple reactions, prepare a master mix of components common to all and then dispense appropriate volumes into each PCR reaction tube prior to adding template DNA and primers.

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	
10x Reaction Buffer	5 µl	1X
$MgCl_2$	2 µl	2 mM
dNTP (Mix 10 mM)	1 µl	200 µM
Taq DNA Polymerase (5 U/ µl)	0,25 – 0,5 µl	1,25 – 2,5 U/reaction

Mix and briefly centrifuge the components.

2. Add template DNA and primers

Components	50 µL rxn	[final]
Foward primer (10 µM)	0,5 - 2,5 µl	0,1 – 0,5 µM
Reverse primer (10 µM)	0,5 - 2,5 µl	0,1 – 0,5 µM
DNA template		10 pg – 1 µg**

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

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

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3. Optimization of $MgCl_2$ concentration:

A recommended concentration for most applications is 2 mM. For an individual optimization add $MgCl_2$ stock solution as shown in the table below.

$MgCl_2$ Final Concentration	2,5 mM	3 mM	4 mM
$MgCl_2$ stock volume to 50 µl	2.5 µl	3 µl	4 µl

4. Incubate reactions in a thermal cycler.

Recommended cycling conditions:

Step		Temp.	Time
Initial denaturation		95 °C	1 - 3 min
30 cycles	Denaturation	95 °C	15 - 30 sec
	Annealing [1]	45-68 °C	15 - 30 sec
	Elongation [2]	72 °C	1 min/kbp
Final extension (optional)		72 °C	1 - 2 min/kbp
Hold		4 - 8 °C	

1)The annealing temperature depends on the melting temperature of the primers used.

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.