DATA SHEET

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Taq Pol Hot Start - Ab+ (-) Mg^{+2}

Heat-activatable DNA polymerase for high specificity, antibody-blocked

Thermus aquaticus, recombinant, *E. coli*

Cat. Nº.	Amount
☐ POL-126XS	250 units
☐ POL-126S	500 units
☐ POL-126M	1.000 units
☐ POL-126L	2 x 1.000 units (M)
☐ POL-126XL	4 x 1.000 units (M)
☐ POL-126XXL	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

Kit contents:

Taq Pol Hot Start - POL-123 (blue cap)
5 units/µl Taq Pol Hot Start in Tris-HCl pH 8.0 (25°C), KCl, EDTA, DTT, 50% (v/v) Glycerol and stabilizers.

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

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

Description:
Hot Start Pol provides improved specificity and sensitivity when amplifying low-copy-number targets in complex backgrounds or when prolonged room-temperature set up is required. The polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of nonspecifically annealed primers and primer-dimer formation at low temperatures during PCR setup. The enzyme 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. The engineered Taq DNA Pol hot start enzyme allows amplification of fragments up to 5 kbp.

Activation step:
Hot Start Pol does not require a prolonged heating or denaturing step. The polymerase inhibiting antibody is quickly released at the increased temperature of the hot start cycle.

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
dNTP (Mix 10 mM)	1 µL	200 µM
Taq DNA Polymerase (5U/ µL)	0,25-0,5 µl	1,25-2,5 U/reaction
$MgCl_2$	2 µl	2 mM

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:

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	3,0 µl	4,0 µl

4. Incubate reactions in a thermal cycler.

Recommended cycling conditions:

Step		Temp.	Time
Initial denaturation		95 °C	2 - 5 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
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.

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