DATA SHEET





Taq High Fidelity Pol

Thermostable DNA polymerase for high accuracy Thermus and Pyrococcus species, recombinant, E. coli

Cat. N°.	Amount
■ POL-106XS	250 units
□ POL-106S	500 units
□ POL-106M	1.000 units
□ POL-106L	2 x 1.000 units (M)
□ POL-106XL	4 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 acidinsoluble 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:

36 months

Kit contents:

Taq High Fidelity Pol (blue cap)

5 units/µl Taq High Fidelity Pol in Tris-HCl, KCl, EDTA, DTT, 50% (v/v) Glycerol, pH 8.0 (25°C) and stabilizers.

Taq High Fidelity Pol Reaction Buffer complete (red cap) -

Tris-HCl pH 9,0 (25°C), KCl, 15 mM MgCl₂.

Description:

Taq High Fidelity Pol is based on a blend of Taq DNA polymerase and a proofreading enzyme specially designed for highly accurate and efficient amplification. It shows excellent results with extremely long (up to 25 kb), GC-rich or other difficult templates. The enzyme blend includes a highly processive $5' \rightarrow 3'$ DNA polymerase and possesses a 5' 3' polymerizationdependent exonuclease replacement activity. Its inherent 3'→5' exonuclease proofreading activity results in a greatly increased fidelity of DNA synthesis compared to Taq polymerase. The enzyme is highly purified and free of bacterial DNA.

Fidelity of the enzyme:

Taq High Fidelity Pol is characterized by a 2-fold higher fidelity compared to Taq polymerase.

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 μΙ	200 μΜ
Taq High fidelity DNA Pol (5U/ μl)	0,25 - 0,5µl	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 μΙ	0,1 – 0,5 μΜ
Reverse primer (10 µM)	0,5 - 2,5 μΙ	0,1 – 0,5 μΜ
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. Incubate reactions in a thermal cycler.

Recommended cycling conditions:

	Step	Temp.	Time
Initial denaturation		95 °C	1 min - 3 min
25-35 cycles	Denaturation	95 °C	15 - 30 sec
	Annealing ¹	45-70 °C	15 - 30 sec
	Elongation ²	72 °C	1 min/kb
Final extension (Optional)		72 °C	1-2 min/kb
Hold		4 - 8 °C	

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

2)The elongation time depends on the length of the fragments to be amplified. A

Note: For large fragments it is recommended in the elongation step to use 68 °C and 1,5 - 2 min/kbp.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary for each new template DNA and/or primer pair.

