DATA SHEET

cellco

Pfu Pol

Proofreading DNA polymerase for highest accuracy, *Pyrococcus furiosus*, recombinant, *E. coli.*

Cat. Nº.	Amount
■ POL-104XS	250 units
□ POL-104S	500 units
□ POL-104M	1.000 units
□ POL-104L	2 x 1.000 units (M)
□ POL-104XL	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 acid-insoluble form in 30 minutes at 70 °C.

Concentration:
2,5 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:

***Pfu* (blue cap)**
2,5 units/µl Pfu in 20 mM Tris-HCl pH 8.0 (25°C), KCl, EDTA, DTT, 50% (v/v) Glycerol and stabilizers.

***Pfu* Reaction Buffer complete (red cap) - 10x**
Tris-HCl pH 8,8 (25°C), KCl and 25 mM $MgCl_2$.

Description:
Pfu Pol is a thermostable proofreading enzyme specially designed for highly accurate and efficient amplification it replicates into dsDNA at 72 °C . It catalyzes the polymerization of nucleotide in the 5' → 3' direction in the presence of magnesium and exhibits a 3' → 5' exonuclease (proofreading) activity. This features results in a greatly increased fidelity of DNA synthesis compared to Taq polymerase. The enzyme is highly purified and free of bacterial DNA. The amplification results in blunt-ended fragments.

Fidelity of the enzyme:
Pfu Pol is characterized by a 6x higher fidelity compared to Taq DNA 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 µl	200 µM
Pfu Pol (2,5U/ µl)	0,5 µl	1,25 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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4. Incubate reactions in a thermal cycler.

Recommended cycling conditions:

Step		Temp.	Time
Initial denaturation		98 °C	30 sec
25-35 cycles	Denaturation	98 °C	5 - 10 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 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.