

Pfu-X Polymerase

Proofreading DNA polymerase for highest accuracy

Pyrococcus furiosus, recombinant, *E. coli*

	Cat. No.	Size	Conc.
	PCR-207S	100 units	2.5 units/μl
	PCR-207L	500 units	2.5 units/μl

For *in vitro* use only
 Quality guaranteed for 12 months
 Store at -20 °C, avoid frequent thawing and freezing

Description

Pfu-X Polymerase is the ideal choice for applications where the efficient amplification of DNA with highest fidelity is required.

The enzyme is a genetically engineered Pfu DNA polymerase, but showing a 2-fold higher accuracy and an increased processivity, resulting in shorter elongation times.

The enzyme catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction and possesses a 5'→3' polymerization-dependent exonuclease replacement activity. Its inherent 3'→5' exonuclease proofreading activity results in a greatly increased fidelity of DNA synthesis compared to Taq polymerase. Pfu-X Polymerase-generated PCR fragments are blunt-ended.

The enzyme is highly purified and free of bacterial DNA.

Fidelity of the enzyme

Pfu-X Polymerase is characterized by a 50-fold higher fidelity compared to Taq polymerase and a 2-fold higher fidelity compared to standard Pfu polymerase.

$$ER_{\text{Pfu-X Polymerase}} = 0.25 \times 10^{-6}$$

The error rate (ER) of a PCR reaction is calculated using the equation $ER = MF / (bp \times d)$, where MF is the mutation frequency, bp is the number of base pairs of the fragment and d is the number of doublings ($2^d = \text{amount of product} / \text{amount of template}$).

Unit definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmol of dNTP into an acid-insoluble form in 30 minutes at 74 °C.

Recommended PCR assay

50 μl assay			
Pfu-X Buffer	10x	5 μl	green cap
dNTPmix	10 mM each	200 μM	
forward Primer	10 μM	2 μl	
reverse Primer	10 μM	2 μl	
Template DNA		1-100 ng	
Pfu-X Polymerase *	2.5 units/μl	0.5 μl	red cap
PCR grade H ₂ O		fill up to 50 μl	

* Please note that it is essential to add the polymerase last.

Pfu-X Polymerase (red cap)

2.5 units/μl Pfu-X polymerase in storage buffer

Pfu-X Buffer (green cap)

10x conc.

Data Sheet

Recommended thermocycling conditions

Three-step standard protocol

Initial denaturation	95 °C	2 min	1x
Denaturation	95 °C	20 sec	25-30x
Annealing ¹⁾	50-68 °C	30 sec	
Elongation ²⁾	68 °C	30 sec/kb	
Final elongation	68 °C	30 sec/kb	1x

Two-step protocol for amplification of longer fragments (>3 kb)

Please note that for performing two-step cycling a sufficiently high primer T_m is necessary. If T_m of primers is below 65 °C or two-step PCR does not yield a sufficient product quality the three-step cycling protocol is recommended.

Initial denaturation	95 °C	2 min	1x
Denaturation	95 °C	20 sec	25-30x
Annealing / Elongation ^{1,2)}	68 °C	30 sec/kb	
Final elongation	68 °C	30 sec/kb	1x

- 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 30 sec/kb is recommended.

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

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