

Pfu-X Polymerase

Proofreading DNA polymerase for high 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

Pfu-X Polymerase (red cap)

2.5 units/μl Pfu-X DNA polymerase in storage buffer

10x Pfu-X buffer <3 kb (green cap)

10x reaction buffer for fragments up to 3 kb

10x Pfu-X buffer >3kb (blue cap)

10x reaction buffer for fragments larger than 3 kb

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

Fragment size up to 3 kb

50 µl assay, fragments <3kb		
5 µl	Pfu-X buffer <3 kb	green cap
200 µM	each dNTP	
0.2-0.5 µM	forward Primer	
0.2-0.5 µM	reverse Primer	
1-100 ng	Template DNA	
0.5 µl (1.25 units)	Pfu-X Polymerase *	red cap
Fill up to 50 µl	PCR grade H ₂ O	

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

Recommended thermocycling conditions

Fragment size up to 3 kb

Initial denaturation	95°C	2 min	1x
Denaturation	95°C	20 sec	25-30x
Annealing ¹⁾	50-68°C	30 sec	
Elongation ²⁾	72°C	30 sec / kb	
Final elongation	72°C	30 sec / kb	1x

Recommended PCR assay

Fragment size larger than 3 kb

50 µl assay, fragments >3kb		
5 µl	Pfu-X buffer >3 kb	blue cap
300 µM	each dNTP	
0.2-0.5 µM	forward Primer	
0.2-0.5 µM	reverse Primer	
1-100 ng	Template DNA	
0.5 µl (1.25 units)	Pfu-X Polymerase *	red cap
Fill up to 50 µl	PCR grade H ₂ O	

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

Recommended thermocycling conditions

Fragment size larger than 3 kb

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 1 min/kbp is recommended.

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

Related products

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For detailed information please visit
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