



Pfu-X Polymerase

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

Cat. No.	Amount
PCR-207S	100 units
PCR-207L	500 units

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.

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid

Concentration: 2.5 units/µl

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' \rightarrow 3' direction but does not possess a 5' \rightarrow 3' exonuclease replacement activity. Its inherent 3' \rightarrow 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.

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

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 = amount of product / amount of template).

Content:

Pfu-X Pol (red cap)

2.5 units/µl Pfu-X Polymerase in storage buffer

(50 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, stabilizers, 50% Glycerol)

Pfu-X Buffer (green cap)

10x conc.

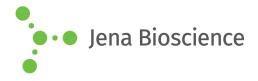
Recommended 50 µl PCR assay:

5 µl	10x Pfu-X Buffer	green cap
200 µM	each dNTP	-
0.4 μΜ	each Primer	-
1 - 100 ng	template DNA	-
0.5 μl (1.25 units)	Pfu-X Pol	red cap
Fill up to 50 µl	PCR-grade water	-

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









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Recommended cycling 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	25-30x		
elongation ²⁾	68 °C	1 min/kb	25-30x		
final elongation	68 °C	1 min/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	25-30x
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/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.

Related Products:

Ready-to-Use Mixes / direct gel loading Ready-to-Use Mixes Thermophilic Polymerases Deoxynucleotides (dNTPs) Supplements Primers and Oligonucleotides DNA Ladders

