interchim

Data sheet

JENA BIOSCIENCE

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.
$ER_{Pfu\text{-}X\ Polymerase} = 0.25x10^{-6}$
The error rate (ER) of a PCR reaction is calculated using the equation ER = MF/(bp x 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).

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_2O	

* 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_2O	

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

For detailed information please visit
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