Pfu-X Core Kit

Proofreading DNA polymerase for high accuracy

Pyrococcus furiosus, recombinant, *E. coli*

Cat.-No.	Size	Conc.
PCR-237S	100 units	2.5 units/µl
PCR-237L	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

dNTP Mix (white cap)
10 mM each dNTP (dATP, dCTP, dGTP, dTTP)

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 Core Kit contains all reagents required for PCR (except template and primer) in one box combining simple handling with high flexibility. The premium quality polymerase, ultrapure dNTPs and the optimized complete reaction buffer ensure superior amplification results.
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 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.

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Recommended PCR assay
Fragment size up to 3 kb

50 µl assay, fragments <3kb		
5 µl	Pfu-X buffer <3 kb	green cap
1 µl	each dNTP	white cap
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
1.5 µl	each dNTP	white cap
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.

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