Data sheet

Pfu-X Polymerase

Proofreading DNA polymerase for highest accuracy

Pyrococcus furiosus, recombinant, *E. coli*

	Cat. No.	Size	Conc.
	PFUX_10KU	10 kU	2.5 units/µl
	PFUX_100KU	100 kU	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 polymerase in storage buffer

Pfu-X Buffer (green cap)
10x conc.

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

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_2O		fill up to 50 µl	

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

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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.