DATA SHEET

LAROVA
The Nucleotide Company

Pfu-X Polymerase

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

Cat. No.	Amount
PFUX_10KU	10 kunits
PFUX_100KU	100 kunits

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 *in vitro* use only!

Shipping: shipped on blue ice

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 polymerisation of nucleotides into duplex DNA in 5'→3' direction but does not possess a 5'→3' 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).

Content:
Pfu-X Pol (red cap)
2.5 units/µl Pfu-X Polymerase in storage buffer
(50 % Glycerol, 50 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT 0.1 % Tween 20, 0.1 % Nonidet P-40)

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

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Recommended 50 µl PCR assay:

5 µl	10x Pfu-X Buffer	green cap
200 µM	each dNTP	-
0.4 µM	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.

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 optimisation of the recommended parameters may be necessary for each new template DNA and/or primer pair.