DATASHEET

FastPfu Fly DNA Polymerase Enzyme

Catalogue No.:abx071020

FastPfu Fly DNA Polymerase is a fast, high fidelity and high processivity hot start DNA polymerase with an extension rate of up to 6 kb/min. Amplification of genomic DNA fragment up to 15 kb. Amplification of plasmid DNA fragment up to 20 kb.

Contents:

Component	**250 U**	**500 U**	**3 kU**
FastPfu Fly DNA Polymerase	1 × 250 U	1 × 500 U	6 × 500 U
5X FastPfu Fly Buffer	1 × 1.2 ml	2 × 1.2 ml	12 × 1.2 ml
50 mM $MgSO_4$	200 µl	400 µl	1 ml
PCR Stimulant	200 µl	400 µl	1 ml
6X DNA Loading Buffer	1 × 500 µl	1 × 1 ml	2 × 1 ml

Target: FastPfu Fly DNA Polymerase

Tested Applications: PCR

Purity: > 99% (SDS-PAGE)

Conjugation: Unconjugated

Storage: Store at -20°C for up to 2 years. Avoid repeated freeze/thaw cycles.

Biological Activity: One unit of FastPfu Fly DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74 °C.

Endotoxin Level: Functional absence of double and single stranded endonuclease activity.

Concentration: 2.5 U/µl

Buffer: FastPfu Fly DNA Polymerase: 50 mM Tris-HCl (pH 8.2), 0.1 mM EDTA, 1 mM DTT, Stabilizers, 50% glycerol.
FastPfu Buffer: 100 mM Tris-SO4 (pH 9.2), 50 mM (NH4)2SO4, 200 mM KCl, 10 mM MgSO4, 10% glycerol.

Directions for use:

Reaction Components:

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 µM)	1 µl	0.2 µM
Reverse Primer (10 µM)	1 µl	0.2 µM
5X FastPfu Fly Buffer	10 µl	1X
2.5 mM dNTPs (not included)	4 µl	0.2 mM
FastPfu Fly DNA Polymerase	1 µl	2.5 U
ddH2O	Variable	N/A
Total Volume	50 µl	N/A

Suggested Reaction Conditions:

Parameter	Target ≤ 10 kb	Target ≥ 10 kb	cDNA Target
Template	100 ng Genomic DNA 5-30 ng Plasmid DNA	200-500 ng Genomic DNA 5-30 ng Plasmid DNA	1-2 µl cDNA from RT reaction (50-500 ng starting RNA template)
MgSO4	Add 1-2 µl of 50 mM MgSO4 to a final concentration of 3-4 mM for targets larger than 5 kb		

Thermal Cycling Conditions:

Number of Cycles	Temperature	cDNA or Genomic DNA	Plasmid DNA
1 cycle	95 °C	2 min	2 min
30-25 cycles	95 °C	20 seconds	20 seconds
(Plasmid/Genomic DNA)	T_M - 5 °C	20 seconds	20 seconds
35-40 cycles (cDNA)	72 °C	6 kb/min for targets ≤ 2 kb 2-4 kb/min for targets > 2 kb	6 kb/min for targets ≤ 6 kb 2-4 kb/min for targets > 6 kb
1 cycle	72 °C	5 min	5 min

Notes:

For GC-rich templates, the recommended denaturation temperature is 98 °C.
To ensure high fidelity, we recommend using high quality dNTPs. dNTPs containing dUTP cannot be used.

Quality Control: Assayed for amplication efficiency to amplify the p53 gene from 10 ng of human genomic DNA.

Note: This product is for research use only.