P Fast-Pfu Taq DNA Polymerase

Cat. No. ABTGMBP04
Store at: -20°C for two years
Concentration 2.5 units/μl

Description

P Fast-Pfu Taq DNA Polymerase is a fast, high fidelity and high processivity hot start DNA polymerase.The fidelity of P Fast-Pfu Taq DNA Polymerase is 54 times higher than P Page DNA polymerase. P Fast-Pfu Taq DNA Polymerasehas extension rate up to 4 kb/min.

Highlights

- Extension rate is about 2-4 kb/min.
- Fidelity is 54 times higher than P Page DNA Polymerase.
- PCR products can be directly cloned into AllBio Blunt vectors.
- Amplification of genomic DNA fragment up to 15 kb.
- Amplification of plasmid DNA fragment up to 20 kb.

Application

- Rapid amplification of high fidelity PCR
- Blunt-end cloning
- Site-directed mutagenesis

Unit Definition

One unit (U) is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into an acid-insoluble material in 30 minutes at 74°C, with activated salmon sperm DNA used as template.

Quality Control

P Fast-Pfu Taq DNA Polymerase has passed the following quality control assays: functional absence of double- and single-stranded endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of P Fast-Pfu Taq DNA Polymerase has been assayed for amplification efficiency from as little as 10 ng of human genomic DNA.

Storage Buffer

50 mM Tris-HCl (pH 8.2), 0.1 mM EDTA, 1 mM DTT, Stabilizers, 50% glycerol

P Fast-Pfu Taq Buffer with 20 mM $MgSO_4$

100 mM Tris-SO_4 (pH 9.2), 50 mM $(NH4)_2SO_4$, 200 mM KCl, 10 mM $MgSO_4$, 10% Glycerol, others

Kit Contents

Component	100 rxns
P Fast-Pfu Taq DNA Polymerase	250 U×1
P Fast-Pfu Taq Buffer	1.2 ml×1
50 mM $MgSO_4$	50 μl×1
PCR Stimulant	50 μl×1

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PCR Stimulant

For better amplification of GC rich or complex template, we recommend adding PCR Stimulant into PCR reaction. PCR Stimulant is provided at 5X concentration and can be used at 0.5X~2.5X concentration.

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
P Fast-Pfu Taq FastPfu Buffer	10 µl	1X
2.5 mM dNTPs	4 µl	0.2 mM
P Fast-Pfu Taq DNA Polymerase	1 µl	2.5 units
ddH_2O	Variable	-
Total volume	50 µl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	2-4 kb/min	
72°C	5-10 min	

Suggested conditions (50 µl reaction volumes)

Parameter	Targets ≦10 kb	Targets≧10 kb	cDNA Targets
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)
$MgSO_4$	Add 1-2 µl of 50 mM $MgSO_4$ to a final concentration of 3-4 mM for target larger than 5 kb		

Thermal cycling conditions

Number of cycles	Temperature	cDNA or Genomic DNA	Plasmid DNA
1 cycle	95°C	2 min	1 min
Plasmid or Genomic DNA:	95°C	10 sec	20 sec
30-35 cycles	Tm-5°C	10 sec	20 sec
cDNA: 35-40 cycles	72°C	4 kb/min for targets≦1 kb	2 kb/min
		2-4 kb/min for targets>1 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 recommended using high quality dNTPs. dNTPs containing dUTP cannot be used.