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EP240408

## Pfu DNA Polymerase

Cat. no. 4992760 Storage: -30~-15°C for two years. Concentration: 2.5 U/µl Product size

Product components	4992760
Pfu DNA Polymerase	500 U
10× Pfu Buffer	1.8 ml

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#### Introduction

*Pfu* DNA Polymerase is a themostable enzyme with a molecular weight of approximately 90 kDa. It catalyzes the polymerization of nucleotides into duplex DNA in the 5'-3' direction in the presence of magnesium and it also exhibits 3'-5' exonuclease (proofreading) activity, resulting in blunt-ended PCR products without 3' -dA overhanges. *Pfu* DNA Polymerase is recommended for use in PCR and primer extension reactions that require high-fidelity DNA synthesis.

#### Unit Definition

One unit of Pfu DNA Polymerase is defined as the amount that incorporates 10 nmol of dNTPs into acidinsoluble material within 30 min at 74°C with activated salmon sperm DNA as the template/primer.

### Storage Buffer

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

#### Applications

High fidelity DNA amplification, such as DNA sequencing, gene expression & cloning, gene site-directed mutagenesis, SNP, end repairing, *etc*.

#### 10× Pfu Buffer

200 mM Tris-HCl (pH 8.8), 100 mM KCl, 100 mM (NH<sub>a</sub>)<sub>2</sub>SO<sub>4</sub>, 20 mM MgSO<sub>4</sub>, other components.

#### Quality Control

Purity up to standard by SDS-PAGE assay. No exogenous nuclease activity is detected. Single copy gene in human genome could be amplified effectively. No significant activity change when stored at room temperature (15-30°C) for one week.

#### Note

- The extension of *Pfu* DNA Polymerase is about 500-1000 bp/min normally. Because *Pfu* DNA Polymerase with 3'-5' exonulease activity may degrade the primers, it should be added later than other components, and immediately go to PCR step.
- As Pfu DNA Polymerase has superior thermostability compared to Taq DNA Polymerase, for high-GC templates, temperature of denaturation can be increased to 98°C, which doesn't have any influence on Pfu DNA Polymerase's activity.

#### Example

Note: The following example is only for reference, user must set up optimal reaction system according to different reaction conditions such as different templates or primers, *etc*.

1. For 50 µl PCR reaction system: 1 kb fragment of human genomic DNA was amplified (If use different reaction system, please proportionally increase or decrease the amount of reaction components referring to this system).

Template	< 1 µg
Primer 1(10 μM)	1 µl
Primer 2(10 μM)	1 µl
10× <i>Pfu</i> Buffer	5 µl
dNTP Mixture(2.5 mM)	4 µl
<i>Pfu</i> (2.5 U/μl)	0.5-1 μl
ddH <sub>2</sub> O	up to 50 µl
2. PCR cycle set-up:	
94°C 3 min	
24°C 30 sec	
55°C 30 sec } 30	cycles
72°C 2 min <sup>J</sup>	

 $72^\circ\text{C}$  5 min 3. Result detection: Load 5  $\mu\text{I}$  PCR products to agarose gel for detection.