

EP191024

Pfu DNA Polymerase

Cat. no. 4992760 Storage: -20°C

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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The product is used for research only, neither intended for the diagnosis, or treatment of a disease, nor for the food, or cosmetics etc.

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 acid-insoluble 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.

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10× Pfu Buffer

200 mM Tris-HCl (pH 8.8), 100 mM KCl, 100 mM (NH₄) $_2$ SO $_4$, 20 mM MgSO $_4$, other components.

Quality Control

Purity>99% by SDS-PAGE test. 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 for one week.

Note

- 1. 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.
- 2. 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 μΙ
Primer 2(10 μM)	1 μΙ
10× <i>Pfu</i> Buffer	5 μΙ
dNTP Mixture(2.5 mM)	4 μΙ
<i>Pfu</i> (2.5 U/μl)	0.5-1 μΙ
ddH ₂ O	up to 50 μl

2. PCR cycle set-up:

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94°C 3 min

94°C 30 sec

55°C 30 sec

72°C 2 min

72°C 5 min
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3. Result detection: Load 5 μ l PCR products to agarose gel for detection.