Pfu DNA Polymerase

Components	Cat. No.			
	E03-01	E03-02	E03-03	E03-04
Pfu DNA Polymerase (2U/ μl)	125μl	250 μl	500 μl	1,250 μl
2x *Pfu* Reaction Buffer	2x1.25ml	7x1.25ml	14x1ml	25x1ml

Description

Pfu DNA Polymerase is a fast and high-fidelity DNA Polymerase with high amplification efficiency. The Polymerase possesses 5 '-3' DNA Polymerase activity and 3 '-5' exonuclease activity. The enzyme was modified by other high-fidelity enzymes with strong amplification ability, fast amplification speed and high fidelity, which overcame the defects of ordinary Pfu enzyme such as poor amplification ability, low yield and slow amplification speed, and greatly shortened the reaction time. This product can be used for long fragment amplification and the expansion of other complex templates. The 3 'end of the amplified PCR product does not contain "A" base, and can be directly cloned in the flat terminal vector. If T/A cloning is needed, "A" should be added to the end of the PCR product for cloning. This product is suitable for gene cloning, gene point mutation, SNP amplification experiments.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using herring sperm DNA as substrate.

Quality Control

After multiple column purification, the purity was more than 98% by SDS-PAGE. No exogenous nuclease activity was detected. After one month at room temperature, there was no obvious change of activity.

PCR Protocol

The following examples are the conventional PCR reaction system and reaction conditions, which should be improved and optimized according to the different template, primer structure and target fragment size in practice.

1. PCR Reaction System

   All operations should be carried out on ice. After thawing, mix the components thoroughly. After use, please put them back to -20° C in time.

Reagent	Quantity, for 50 μl reaction	Final concentration
2xPfu Buffer (Mg^{2+} plus)	25 μl	1x
10 mM dNTPs	1.5-2.5 μl	0.3-0.5 mM each
Primer I, 10 μM	2 μl	0.4 μM
Primer II, 10 μM	2 μl	0.4 μM
Pfu DNA Polymerase（2.0 U/μl）	0.5-0.75 μl	2.5U/50 μl
Template DNA	variable	<500 ng/50μl
Sterile deionized water	variable	-
Total		50 μl

2. **Mix contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.** When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 μl mineral oil.

3. **Perform 25-35 cycles of PCR amplification as follows:**

Initial Denaturation	98℃	30 sec-3 min
25-35 Cycles	98℃ According to Tm 72℃	10-30 sec 15-30 sec 3-5 kb/min
Final Extension	72℃	5 minutes

4. **Maintain the reaction at 4°C. The samples can be stored at -20°C until use.**

5. **Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.**