ELK Biotechnology

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Pfu DNA Polymerase

Cat. No	Specification	Storage/Shelflife
EQ008-01	**50 µl**	-20C°/2 years
EQ008-02	**100 µl**	-20C°/2 years

Introduction

Pfu DNA Polymerase is a thermostable protein isolated and purified from recombinant E.coli strains containing Pyrococcus furiosus DNA Polymerase gene, with a molecular weight of about 90KD. With 5'→3' polymerase activity and 3'→5' exonuclease activity, it can remove mismatched bases inserted during extension.

Pfu DNA Polymerase is the one with the highest fidelity among the heat-resistant DNA polymerases found so far. The PCR product is blunt-ended and can be directly linked to the blunt-ended cloning vector, or the end is treated with A and then connected to the TA vector

Kit Components

Components	EQ008-01	EQ008-02
Pfu DNA Polymerase (5U/µl)	**50 µl**	**100 µl**
10 × Pfu Buffer	**0.5 ml**	**1 ml**
ddH2O	**1 ml**	**1 ml ×2**
User Manuals	**1 copy**	**1 copy**

Unit definition

The activity of 1 unit (U) of Taq DNA polymerase is defined as using activated salmon sperm DNA as a template/primer at 74°C and 30 minutes to incorporate 10 nmol deoxynucleotides into acid-incompatible substances. The amount of enzyme. Activity detection conditions: 50 mM Tris-Hcl (pH 9.0, 25°C), 50 mM NaCl, 5 mM MgCl2, 0.2 mM each dNTPs (including [3H]-Dttp), 200 µg/ml activated calf thymus DNA and 0.1 mg/ml BSA.

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QC Control

The purity detected by SDS-PAGE is greater than 99%. After detection of no exogenous nuclease activity, PCR method detects no host DNA residues, which can effectively amplify single-copy genes in the human genome.

PCR system components

1. The purity of template DNA: Many residual nucleic acid extraction reagents will affect the PCR reaction, including protease, protein denaturant (such as SDS, guanidine salt), high concentration salt (KAc, NaAc, sodium caprylate, etc.) and high concentration EDTA. The amount of template with low purity (such as the template obtained by boiling method) should not exceed 1/10 of the PCR reaction system (for example, the volume of template added to the 50 μl reaction system should not exceed 5 μl). If the purity of the template DNA is too poor, you can use our PCR product recovery kit (Cat. No. EP005) to purify and concentrate the template DNA. The amount of template purified by our PCR product recovery kit can be as much as 1/2 of the volume of the PCR reaction system.
2. The amount of template DNA: a very small amount of DNA can also be used as a template for PCR, but in order to ensure the stability of the reaction, it is recommended to use more than 104 copies of the target sequence as a template for the 50μl system. Recommended amount of template DNA:

Human genomic DNA	0.05 μg~0.5 μg/50 μl PCR reaction system
E. coli genomic DNA	10 ng~100 ng/50 μl PCR reaction system
λ DNA	0.5 ng~5 ng/50 μl PCR reaction system
Plasmid DNA	0.1ng ~ 10 ng/50 μl PCR reaction system

If you need to use the amplified product as a template for further amplification, you should dilute the amplified product by at least 1,000 to 10,000 times before using it as a template, otherwise smeared bands or non-specific bands may appear.

3. Primer concentration: Generally, the concentration of each primer is 10 μM (50×), and the working concentration is 0.2 μM. Excessive primers may cause non-specific amplification, and too few primers may reduce amplification efficiency.

PCR parameter settings

1. Pre-denaturation: Generally, the pre-denaturation is 94℃, 1~5 min. Too high denaturation temperature or too long time will lose the activity of Taq enzyme.
2. Annealing: Annealing temperature is the key to PCR. Too high temperature may reduce yield, and too low temperature may produce primer dimers or non-specific amplification. It is recommended to try 5℃ lower than Tm for the first time PCR amplification (if the two primers Tm are different, refer to the lower Tm) as the annealing temperature. Generally, the primer synthesis company will provide the Tm of the synthesized primer, and the primer

Tm can also be estimated according to this formula: Tm = 2℃×(A+T) + 4℃× (G+C). The optimal annealing temperature needs to be determined by gradient PCR.

3. Extension: The extension temperature is usually 72°C, and the extension time depends on the length of the target DNA fragment. The required extension time is calculated at 500 bp/min time, too long may cause non-specific increase. After the cycle is over, continue to extend for 5-10 minutes to obtain a complete double-stranded product.

3. Number of cycles: 25~35 cycles are generally used, and the number of cycles can be appropriately increased for low-copy templates. However, too many cycles may increase non-specific amplification, but not specific products.

Instructions

1. Thaw 10×Pfu Buffer, dNTPs, ddH2O, template DNA and primers at room temperature and place on ice.

2. Turn the thawed components upside down and mix them evenly, and add them to each group in sequence according to the following table to make a PCR reaction system:

Component	Volumn（μL）
ddH2O	41.5-n
10×Pfu Buffer	5
primer1（10 μM）	1
primer2（10 μM）	1
dNTPs （10 mM each）	1
Pfu DNA Polymerase	0.5
Template DNA	n
Total	50

*10×Pfu Buffer must be mixed well before use, otherwise it will affect the PCR effect.

*Pfu DNA Polymerase must be added after adding dNTPs, otherwise Pfu DNA Polymerase may activate 3'→5' exonuclease activity without dNTPs and begin to degrade primers.

The above examples are the components added to the 50 μl reaction system. If you need a reaction system with other volumes, please increase or decrease the components in proportion.

3.Flick the PCR reaction tube with your fingers to mix thoroughly, and centrifuge at low speed for a few seconds to allow the solution to settle to the bottom of the tube.

4.PCR Example of reaction cycle setup

Cycle steps	Temperature (℃)	Time	cycle number
Pre-denaturation	94	3 min	1
Denaturation	94	30 sec	30
Annealing*	50-60	30 sec	
Extension※	72	30 sec/kb	
Final Extended	72	5 min	1

*Subject to the actual best annealing temperature

※ Calculated at 500bp/min.

5.Result detection: Take 5-10 μl of the amplified product directly for agarose electrophoresis detection.

* The relationship between the concentration of agarose gel and the best resolution range of linear DNA:

Agarose concentration	Best linear DNA resolution range
0.5%	1,000~30,000
0.7%	800~12,000
1.0%	500~10,000
1.2%	400~7,000
1.5%	200~3,000
2.0%	50~2,000