AffiCHEM® Taq DNA Polymerase

Cat.No.	Size	Storage/Shelf life
AFG-EEM-0002	**100 µl/500U**	-20C°/two years
AFG-EEM-0002	**500 µl/2500U**	-20C°/two years

Introduction

Taq DNA Polymerase is a thermostable protein isolated and purified from a recombinant E.coli strain containing Thermus aquaticus DNA Polymerase gene, with a molecular weight of about 90KD. It has 5'→3' polymerase activity and double-strand specific 5'→3' exonuclease activity, but no 3'→5' exonuclease activity.

The Taq DNA Polymerase PCR product is a 3’ single A sticky end, which can be directly connected to the TA vector.

Kit Components

Components	500 U	2500 U
Taq DNA Polymerase (5U/µl)	**100 µl**	**500 µl**
10 × PCR Buffer(Mg2+)	**1 ml**	**5 ml**
ddH2O	**1 ml ×2**	**10ml**
User manual	**1 copy**	**1 copy**

Unit definition

The amount of enzyme required to mix 10 nm dNTPs into acid-insoluble precipitates at 74°C for 30 minutes is defined as 1 activity unit.

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.

QC

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. 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 Circle Purification Kit to purify and concentrate the template DNA. The amount of template purified by our PCR Circle Purification 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 PCR template, but in order to ensure the stability of the reaction, it is recommended to use a 50μl system. 10^4 Copy the above target sequence as a template.

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 re-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×PCR Buffer(Include 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 as shown in the table below to make a PCR reaction system:

Component	Volumn（μL)
ddH2O	41.5-n
10×PCR Buffer	5
primer1（10 μM)	1
primer2（10 μM)	1
Taq DNA Polymerase	0.5
Template DNA	n
Total	50

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

The above examples are the components added in a 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 step	Temperature (℃)	Time	Cycle number
Pre-denaturation	94	5 min	1
Denaturation	94	30 sec	35
Annealing*	50-60	30 sec	
Extended※	72	30 sec/kb	
Final extended	72	10 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	Optimal range of linear DNA resolution
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