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Taq DNA Polymerase

Composition

Cat. No.	8004050	8004250
Taq DNA Polymerase (5U/μl)	50 μl	250 μl
10×PCR Buffer(Mg^{2+})	0.5 ml	1 ml ×3
ddH_2O	1 ml	1 ml ×3
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Storage

It can be stored at -20°C for more than two years.

Introduction

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

The Taq DNA Polymerase PCR product is a 3' single A-sticky end that can be attached directly to the TA vector.

Active Unit

At 74°C for 30 min, the amount of enzyme required to incorporate 10 nm dNTPs into the acid-insoluble precipitate was defined as 1 activity unit.

Activity assay conditions: 50 mM Tris-HCl (pH 9.0, 25°C), 50 mM NaCl, 5 mM $MgCl_2$, 0.2 mM each dNTPs (including [3H]-dTTP), 200 μg/ml activated calf thymus DNA and 0.1 mg/ml BSA.

Quality Control

The purity of the SDS-PAGE assay is greater than 99%. No foreign nuclease activity was detected, and no host DNA residue was detected by PCR method, which could effectively amplify single-copy genes in the human genome.

Components of the PCR system

1. Purity of template DNA: Many residual nucleic acid extraction reagents can affect the PCR reaction, including proteases, protein denaturants (e.g., SDS, guanidine salts), high concentrations of salts (KAc, NaAc, sodium caprylate, etc.), and high concentrations of EDTA. Do not use more than 1/10 of the PCR reaction for less pure templates (e.g., no more than 5 μl of the sample for 50 μl). If the template DNA purity is too poor, the DNA Purification Kit to purify and concentrate the template DNA, and the amount of template used after purification can be as much as 1/2 of the volume of the PCR reaction system.
2. Template DNA dosage: A very small amount of DNA can also be used as a PCR template, but to ensure the stability of the reaction, it is recommended to use more than 10^4 copies of the target sequence as a template for the 50 μl system. Recommended dosage 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：	0.1 ng ~ 10 ng/50 μl PCR reaction system

   If the amplification product is to be used as a template for reamplification, the amplification product should be diluted at least 1,000 to 10,000-fold before using it as a template, otherwise smeared bands or non-specific bands may occur.
3. Primer concentration: Typically, each primer is prepared at a concentration of 10 μM (50×) and a working concentration of 0.2 μM. Too much primer may result in non-specific amplification, and too little primer may reduce amplification efficiency.

PCR parameter settings

1. Pre-denaturation: The general pre-denaturation is 94°C, 1~5 min. Denaturation temperatures that are too high or too long can result in loss of Taq enzyme activity.
2. Annealing: Annealing temperature is key for PCR, as too high a temperature may reduce yield, and too low a temperature may result in primer-dimers or non-specific amplification. For the first attempt at PCR

amplification, it is recommended to try a lower than 5°C Tm (if the two primer Tm are different, refer to the lower Tm) as the annealing temperature. Generally, primer synthesis companies will provide the Tm of the synthesized primers, and the primer Tm can also be estimated according to this formula: Tm = 2°C×(A+T) + 4°C× (G+C). The optimal annealing temperature needs to be determined by gradient PCR.

3. Extension: The extension temperature is typically 72°C, and the length of extension time depends on the length of the DNA fragment of interest, and the desired extension time is calculated at 1 kb/min, which may result in a non-specific increase. After the end of the cycle, continue to extend for 5~10 min to obtain the complete double-stranded product.
4. Number of cycles: Generally, 25~35 cycles are used, and the number of cycles can be increased appropriately with a low copy template. However, excessive number of cycles may increase non-specific amplification without increasing specific products.

Protocol

1. Thaw 10× PCR Buffer (Mg^{2+}), dNTPs, ddH_2O, template DNA, and primers at room temperature and place on ice.
2. Invert the thawed components to mix evenly, and add each component in turn as shown in the following table to prepare a PCR reaction system:

ddH_2O	(41.5-n) µl
10×PCR Buffer(Mg^{2+})	5 µl
Primer 1(10 µM)	1 µl
Primer 2(10 µM)	1 µl
dNTPs（10 mM each）	1 µl
Taq DNA Polymerase	0.5 µl
template	n µl
Total	50 µl

Note:

- 10×PCR Buffer (Mg^{2+}) must be well mixed before use, otherwise the PCR effect will be affected.
- The above example is the addition of components for a 50 µl reaction, if additional volumes of the reaction are required, increase, or decrease the components proportionally.

3. Flick your finger on the PCR reaction tube to mix well, and spin down the solution to the bottom of the tube.
4. Example of PCR reaction cycle setup

94°C 3 min
94°C 30 sec
※55°C 30 sec
§ 72°C 1 min } 30 Cycles
72°C 5 min

※The actual optimal annealing temperature shall prevail.

§ Calculated at 1 kb/min.

5. Results: 5-10 µl of the amplified product was mixed with Loading Buffer and then detected by agarose gel electrophoresis.

Relationship between agarose gel concentration and the optimal resolution range of linear DNA:

Agarose	Optimal 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