Taq DNA Polymerase (Mg^{2+} plus buffer)

P101

Version 23.1

Product Description

This product is expressed by *Escherichia coli* cloned with *Thermus aquaticus* DNA Polymerase gene, and then refined through multi-step purification. It does not contain endonuclease, exonuclease and bacterial DNA. Taq DNA Polymerase has 5'→3' polymerase activity and 5'→3' exonuclease activity, but no 3'→5' exonuclease activity. The PCR product has an adenine at the 3' end that can be cloned into the T vector. It is also compatible with ClonExpress and TOPO cloning kits (Vazyme #C112/C113/C115).

Components

Components	P101-01 (1,000 U)	P101-03 (10,000 U)
10 × Taq Buffer (Mg^{2+} plus)	4 × 1 ml	10 × P101-01
Taq DNA Polymerase (5 U/μl)	200 μl	

Storage

Store at -30 ~ -15℃ and transport ≤0℃.

Applications

It is applicable for DNA amplification reactions of animal, plant and microbial.

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble material in 30 min at 74℃, with activated salmon sperm DNA as the template/primer.

Notes

For research use only. Not for use in diagnostic procedures.

Operation precautions

Taq DNA Polymerase has a certain reactivity at room temperature, please prepare the PCR System on ice, and then place it on the PCR machine for the reaction. This can reduce nonspecific amplification during the reaction preparation stage and help to obtain highly specific amplification results.

Primer Design Guidance

1. It is recommended that the last base at the 3' end of the primer should be G or C.
2. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer.
3. Avoid hairpin structures at the 3' end of the primer.
4. Differences in the Tm value of the forward primer and the reverse primer should be no more than 1℃ and the Tm value should be adjusted to 55 ~ 65℃ (Primer Premier 5 is recommended to calculate the Tm value).
5. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value.
6. It is recommended that the GC content of the primer to be 40% - 60%.
7. The overall distribution of A, G, C, and T in the primer should be as even as possible. Avoid using regions with high GC or AT contents.
8. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers. Avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
9. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

Experiment Process

Reaction System

Components	Volume
ddH_2O	To 50 µl
10 × Taq Buffer (Mg^{2+} plus)	5 µl
dNTP Mix (10 mM each)	1 µl
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
Template DNA[a]	x µl
Taq DNA Polymerase (5 U/µl)[b]	0.5 µl

▲ When the GC content of amplified fragment is >60% and the optimized conditions cannot be amplified normally. It is recommended to use PCR Enhancer to optimize the amplification reaction.

a. Optimal reaction concentration varies in different templates. In a 50 µl system, the recommended template usage is as follows:

Animal & Plant Genomic DNA	0.1 - 1 µg
Escherichia coli Genomic DNA	10 - 100 ng
λDNA	0.5 - 10 ng
cDNA	1 - 5 µl (≤1/10 of the total volume of PCR system)
Plasmid DNA	0.1 - 10 ng

b. The amount of Taq DNA Polymerase can be adjusted between 0.25 - 1 µl. Increasing the amount of Taq DNA polymerase can improve the amplification yield under normal circumstances, but may decrease the specificity of PCR amplifcation.

Reaction Program

Temperature	Time	Cycle number
95℃	3 min (Initial Denaturation)[a]	
95℃	15 sec	30 - 35 cycles
60℃[b]	15 sec	
72℃	60 sec/kb	
72℃	5 min (Final Extension)	

a. The condition of initial denaturation is applicable for most amplification reactions and can be adjusted according to the complexity of the template structure. If the template structure is complex, the initial denaturation time can be extended to 5 - 10 min to improve its effect.

b. The annealing temperature needs to be adjusted according to the Tm value of the primer, generally set to be 3 ~ 5℃ lower than the Tm value of the primer; For complex templates, it is necessary to adjust the annealing temperature and extend the extension time to achieve efficient amplification.