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

Catalog # P102

Version 5.1

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Introduction

Taq DNA Polymerase is a thermostable DNA polymerase that exhibits a 5 '→3 'polymerase activity and a 5 '→3 'exonuclease activity, with no 3 '→5 'exonuclease activity. Taq DNA Polymerase is purifed from an Escherichia coli (*E.coli*) strain overexpressing the gene of Thermus aquaticus DNA Polymerase. No endonuclease, exonuclease, or bacterial DNA were detected in this kit.

The PCR products contain A at the 3'-end and can be directly cloned into T-Vectors. The products are also compatible with Vazyme ClonExpress II One Step Cloning Kit series (Vazyme, C#C112, #C113).

Package Information

Components	P102-01 1,000 U	P102-d1 1,000 U	P102-02/d2 5,000 U	P102-03/d3 10,000 U
10× Taq Buffer (Mg^{2+} free)	4 ml	4 ml		
25 mM $MgCl_2$	4 ml	4 ml		
dNTP Mix (10 mM each)	------	800 µl		
Taq DNA Polymerase (5 U/µl)	200 µl	200 µl	P102-01/d1 × 5	P102-01/d1 × 10

Storage

Store at -20℃.

Unit Definition

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

Quality Control

Exonuclease Activity: The product is tested in a reaction containing 10 U of Taq DNA Polymerase and 0.6 µg of λ-Hind III. After incubation at 37℃ for 16 hours, there is no visually discernible change to DNA bands determined by agarose gel electrophoresis.

Endonuclease Activity: The product is tested in a reaction containing 10 U of Taq DNA Polymerase and 0.6 µg of Supercoiled pBR322. After incubation at 37℃ for 4 hours, there is no visually discernible change to DNA band determined by agarose gel electrophoresis.

Functional Assay: The human α-1-antitrypsin gene is amplified for 30 cycles in a 50 µl system using 1.25 U of Taq DNA Polymerase and 100 ng human genomic DNA as template. A single DNA band of 360 bp is detected by 1% agarose gel electrophoresis.

Protocol

1. General reaction mixture for PCR:

10× Taq Buffer (Mg^{2+} free)	5 µl
25 mM $MgCl_2$ [a]	optional
dNTP Mix (10 mM each)	1 µl
Template DNA [b]	optional
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
Taq DNA Polymerase (5 U/µl) [c]	0.5 µl
ddH_2O	to 50 µl

a. The final concentration of Mg^{2+} of this mixture is 2 mM, as for most PCR reactions, the optimized volume of 25 mM $MgCl_2$ in a 50 µl PCR system is 3 µl-4 µl. However, if necessary, the concentration of Mg^{2+} can be increased by adding 25 mM $MgCl_2$.

b. The recommended amount of DNA template for a 50 µl reaction system is as follows:

Human Genomic DNA	0.1 - 1 µg
Bacterial Genomic DNA	10 - 100 ng
λ DNA	0.5 - 5 ng
Plasmid DNA	0.1 - 10 ng

c. The amount of Taq DNA Polymerase can be adjusted between 0.25 µl and 1 µl. Generally, higher level of Taq will increase the yield of PCR products but may decrease the specificity of PCR amplifcation.

2. Thermocycling conditions for a routine PCR:

94℃	5 min (Pre-denaturation)	
94℃	30 sec	30 - 35 cycles
55℃*	30 sec	
72℃	60 sec / kb	
72℃	7 min (Final extension)	

*The optimal annealing temperature should be 1℃-2℃ lower than the T_m of the primers used.

Handling Notes

Taq DNA Polymerase also shows polymerase activity at room temperature. Therefore, it is recommended to set up reaction systems on ice and then immediately start the reaction in a PCR amplifier, so as to reduce nonspecific amplification during preparation and get better PCR results.

Primers Designing Notes

1. Choose C or G as the last base of the 3'-end of the primer;
2. Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer;
3. Avoid hairpin structure at the 3'-end of the primer;
4. T_m of the primers should be within the range of 55℃ - 65℃;
5. Additional sequence should not be included when calculating Tm of the primers;
6. GC content of the primers should be within the range of 40% - 60%;
7. T_m and GC content of forward and reverse primes should be as similar as possible.