ACE Taq-Plus DNA Polymerase

Cat# EP1105 – 250U | EP1106 – 1000U | EP1107 – 3000U

Storage: All components should be stored at -20℃.

INTRODUCTION

Taq Plus DNA polymerase is a mixture of Taq DNA polymerase and an enzyme containing 3'→5' exonuclease activity. The fidelity of Taq Plus is 6 times greater than that of Taq DNA Polymerase. Compared with Taq DNA Polymerase, Taq Plus DNA polymerase has stronger amplification performance, higher sensitivity and yield, and is more tolerant of impurities within 5 kb amplifying range. The obtained PCR products are compatible with CE One Step Cloning Kit series (ACE, Cat.No. #EC1001, #EC1008). The PCR products contain A at the 3'-end and can be directly cloned into T-Vectors.

CONTENTS

Component	EP1105 250 U	EP1106 1000U	EP1107 3000U
10× Taq Plus Buffer (Mg^{2+} plus)	1ml	4ml	4ml*3
Taq Plus DNA Polymerase (5 U/μl)	50ul	200ul	200ul*3

UNIT DEFINITION

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble products in 30 min 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 and0.6μgof 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 and0.6μgofSupercoiledpBR322. 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-antitrypsingeneisamplifiedfor30cyclesina50μlsystemusing1.25Uof 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:

ddH2O	to 50 µl
10× Taq Plus Buffer (Mg2+ plus)	5 µl
25 mM $MgCl_2$ [a]	Optional
dNTP Mix (10 mM each)	1 µl
Template DNA[b]	optional
Primer1(10µM)	2µl
Primer2(10µM)	2µl
Taq Plus DNA Polymerase(5U/µl) [c]	0.5µl

a. The final concentration of Mg^{2+} of this mixture is 2 mM, as for most PCR reactions, the optimized final concentration of Mg^{2+} is 1.5 mM- 2 mM. 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 0.25µl and 1µl. 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. Tm 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. Tm and GC content of forward and reverse primes should be as similar as possible.

PRODUCT USE LIMITATION

These products are intended for research use only.