ACE Super-Fidelity DNA PCR kit

Cat# EP1005 – 100U / EP1006 - 500U

Storage at -20 ℃ for one year

INTRODUCTION

ACE Super-Fidelity DNA Polymerase is a new generation superior enzyme based on Pfu DNA Polymerase for robust PCR with extreme fidelity. High amplification efficiency and template adaptability make ACE Super-Fidelity DNA Polymerase suitable for almost all PCR reactions.

The unique extension factor, specificity-promoting factors and plateau-inhibiting factor in ACE Super-Fidelity DNA Polymerase greatly improve its long-fragment amplification ability, specificity, and yield.

ACE Super-Fidelity DNA Polymerase is capable of amplifying long fragments such as 40 kb λDNA, 20 kb genomic DNA and 10 kb cDNA. The amplification error rate of ACE Super-Fidelity DNA Polymerase is 53-fold lower than that of Taq and 6-fold lower than that of Pfu. In addition, ACE Super-Fidelity DNA Polymerase has a good resistance to PCR inhibitors and can be used for direct PCR amplifications with bacteria, fungi, plants, animal tissues, and even whole blood samples. ACE Super-Fidelity DNA Polymerase contains two monoclonal antibodies inhibiting the 5′ → 3′ polymerase activity and 3′ → 5′ exonuclease activity at room temperature. Super fidelity and supreme amplification efficiency make ACE Super-Fidelity DNA Polymerase the best choice for high fidelity PCR. Amplification will generate blunt-ended products, which are compatible with other cloning Kit

CONTENTS

No	Component	**EP1005 – 100 U**	**EP1006– 500 U**
DA	ACE Super-Fidelity DNA Polymerase (1 U/ul)	100 µl	100 µl x 5
DB	2X ACE Super-Fidelity Buffer	1.25 ml x 2	1.25 ml x 10
DC	10 mM dNTP Mix	100 µl	100 µl x 5
DD	10X Loading buffer	1.25 ml	1.25 ml x 5

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.

PROTOCOL

1. Mix the following components

ACE Super-Fidelity DNA Polymerase (1 U/ul) [a]	1 ul
2X ACE Super-Fidelity Buffer [b]	25 ul
10 mM dNTP Mix	1 ul
Primer 1 (10 uM)	2 ul
Primer 2 (10 uM)	2 ul
Template DNA / cDNA [c]	Optional
ddH_2O	To 50 ul

a. The recommended final concentration of ACE Super-Fidelity DNA Polymerase is 1 U/50 µl, which can be optimized between 0.5 U/50 µl and 2 U/50µl, if necessary.

b. 2X ACE Super-Fidelity Buffer contains Mg^{2+}. The final concentration of Mg^{2+} is 2 mM.

c. Optimal reaction concentration varies from templates. In a 50 µl system, there commended template usage is as follows: genomic DNA, 50 ng-400 ng; plasmid or virus DNA, 10 pg-30 ng; cDNA, 1 µl-5 µl.

2. Place the sample in a PCR instrument and run the following program for PCR:

Stage	Temp.	Time	Cycle
Pre-Denaturation[a]	95℃	30 s / 3 min	1
Denaturation	95℃	15 s	25-35
Annealing[b]	56-72℃	15 s	
Extension[c]	72 ℃	30-60s/kb	
Final Extension	95℃	15 s	1

a. For pre-denaturation, the recommended time is 30 sec for plasmid/virus DNA and 3 min for genomic DNA / cDNA.

b. For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72℃, the annealing step can be removed (two-step PCR). If necessary, the annealing temperature can be further optimized in a gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature in 3℃ increments is helpful to improve poor amplification specificity.

c. Longer extension time is helpful to increase the amplification yield.

For Long-fragment PCR

Use long primers.

When the recommended PCR program does not work, try the Touch Down Two-step PCR as follows:

Stage	Temp.	Time	Cycle
Pre-denaturation	95℃	30 s / 3 min	1
Denaturation	95℃	15 s	5
Extension	74℃	60 s /kb	
Denaturation	95℃	15 s	5
Extension	72℃	60 s / kb	
Denaturation	95℃	15 s	5
Extension	70℃	60 s / kb	
Denaturation	95℃	15 s	25
Extension	68℃	60 s / kb	
Final Extension	68℃	5 min	1

Primer Design 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. The 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 primers should be as similar as possible.

PRODUCT USE LIMITATION

These products are intended for research use only.