Cyrusbioscience

Excel™ Super-Fidelity DNA Polymerase

Product No.: VZ-505P

Introduction

Excel™ Super-Fidelity DNA Polymerase is a new generation superior enzyme based on Excel™ DNA Polymerase for robust PCR with higher fidelity. The unique extension factor, specificity-promoting factors and plateau-inhibiting factor newly added to Excel™ Super-Fidelity greatly improve its long-fragment amplification ability, specificity, and PCR yield. P Excel™ Super-Fidelity is capable of amplifying long fragments such as 40 kb λ DNA, 40 kb plasmid DNA, 20 kb genomic DNA and 10 kb cDNA. The amplification error rate of Excel™ Super-Fidelity is 53-fold lower than that of conventional Taq and 6-fold lower than that of Pfu. In addition, Excel™ Super-Fidelity has a good resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Excel™ Super-Fidelity contains two monoclonal antibodies inhibiting the 5'->3' polymerase activity and 3'->5' exonuclease activity at room temperature, which enable Excel™ Super-Fidelity to perform greatly-specific Hot-Start PCRs. The amplification generates blunt-ended products, which need additional A-tailing step for compatible with general TA cloning kit.

Components

	100 U	500 U	1000 U
Excel Super-Fidelity DNA Polymerase (1 U/μl)	100 μl	5× 100 μl	10× 100 μl
2× Excel Super-Fidelity Buffer	1.25 ml	5× 1.25 ml	10× 1.25 ml
dNTP Mix (10 mM each)	100 μl	5× 100 μl	10× 100 μl
10× Loading buffer	1.25 μl	5× 1.25 μl	10× 1.25 μl

Storage: Store at -20℃; avoid repeated freezing and thawing.

Unit Definition

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

Quality Control

Residual Endonulease Test: Incubate 10 U of this enzyme and 0.3 μg of Supercoiled pBR322 DNA at 37℃ for 4h; the DNA electrophoresis bands remain unchanged.

Residual gDNA Test: Detecting the residual nuclear acid in 10 U of this enzyme with 16S rDNA-specific TaqMan qPCR, the genome DNA of is less than 10 copies.

Function Assay: Load 1 U of this enzyme into a 50 μl PCR system and set extension time as 30 sec/kb, amplify 5 fragments with various lengths and different contents of GC respectively and use 10 ng of λ DNA, 50 ng of plasmid DNA, 100 ng of human genome DNA and 1 ul of cDNA from Hela cells respectively. After 35 cycles, use 1/10 of the PCR products to perform 1% agarose gel electrophoresis and EB staining, then there shall be a specifically single band responding to expect.

Protocol

1. For Conventional PCR

Recommended PCR System

Keep all components on ice during the experiment. All components need to be mixed up thoroughly after thawing and put back to -20℃ immediately for storage after using.

Template DNA[a]	x µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Excel Super-Fidelity DNA Polymerase (1 U/µl)	1 µl
dNTP Mix (10 mM each)	1 µl
2× Excel Super-Fidelity Buffer[b]	25 µl
ddH2O	up to 50 µl

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

Templates	Input Template DNA
Genomic DNA	50 - 400 ng
Plasmid or Virus DNA	10 pg - 30 ng
cDNA	1 - 5 µl (≤ 1/10 of the total volume of PCR system)

b. 2× Excel Super-Fidelity Buffer contains Mg2+. The final concentration of Mg2+ is 2 mM.

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation [a]	95℃	30 sec/3 min	1
Denaturation	95℃	15 sec	25-35
Annealing [b]	56℃ - 72℃	15 sec	
Extension c	72℃	30 - 60 sec / kb	
Final Extension	72℃	5 min	1

a. For pre-denaturation, the recommended temperature is 95℃, and 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, annealing temperature can be further optimized in a gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature is helpful to improve poor amplification specificity.

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

2. For Long-fragment PCR

Excel Super-Fidelity DNA Polymerase can extraordinarily perform a long-fragment amplification with high specificity and yields. If the recommended program is failure to work, the following Touch Down two-step PCR may be helpful:

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	1
Denaturation	92℃	15 sec	5
Extension	74℃	60 sec / kb	
Denaturation	95℃	15 sec	5
Extension	72℃	60 sec / kb	
Denaturation	95℃	15 sec	5
Extension	70℃	60 sec / kb	
Denaturation	95℃	15 sec	25
Extension	68℃	60 sec / kb	
Final Extension	68℃	5 min	1

It is recommended to use high-quality templates and long primers. Increasing the input of template DNA may be helpful to improve the amplification yield.

3. For PCR Using Crude Material as Template

Excel Super-Fidelity has a good resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Crude materials that have been successfully amplified with Excel Super-Fidelity are as follows:

Sample Type	Amplification Method	Template Recommendation (for a 50 µl PCR system)
Whole Blood	Direct PCR	1 - 5 µl
Filter Paper Dry Blood	Direct PCR	1 - 2 mm2 filter paper
Cultured Cells	Direct PCR	Little amount of cells
Yeast	Direct PCR	A monoclone or 1 µl suspension
Bacteria	Direct PCR	A monoclone or 1 µl suspension
Mold	Direct PCR	Little amount of sample
Sperm	Direct PCR	Little amount of sample
Plankton	Direct PCR	Little amount of sample
Plant Tissue	Direct PCR	1 - 2 mm2 tissue
Mouse Tail	PCR with lysate	1 - 5 µl lysate*
Food	PCR with lysate	1 - 5 µl lysate*

* Lysate Preparation:

Animal Tissues Foods → Submerge little mount of samples in lysis buffer with final concentration 200 µg/ml of Proteinase K (self-provide) → 60℃ 10 min, 95℃ 10min → Mix well and spin

at room temperature; Collect the supernatant as lysate

Lysis Buffer: 20 mM of Tris-HCl, 100 mM of EDTA, 0.1% SDS, pH 8.0 (not included in this kit).

Attentions

1. Use high-quality templates.
2. DO NOT use dUTP or any primers or templates that contain uracil.
3. Properly improve the input of Excel Super-Fidelity DNA Polymerase according to the experiment demands, but no more than 2 U in a 50 µl reaction system.
4. The Excel Super-Fidelity DNA Polymerase has strong proofreading activity. Therefore, the PCR products must be purified before adding A-Tailing when TA cloning.
5. To prevent the strong proofreading activity of the Excel Super-Fidelity DNA Polymerase degrading primers, the polymerase should be loaded lastly when making up the reaction system.
6. Primers design notes:

*Choose C or G as the last base of the 3'-end of the primer.

*Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer.

*Avoid hairpin structure at the 3'-end of the primer.

*Tm of the primers should be within the range of 55℃ - 65℃ (recommend to calculate in Primer Premier 5), and the Tm difference between F and R primers should be less than 1℃.

*Additional sequence should not be included when calculating Tm of the primers.

*GC content of the primers should be within the range of 40% - 60%.

*The general distribution of A, G, T, C in the primers should be uniform, and avoid using regions with rich GC and rich AT.

*Keep complementary sequence less than 5 bases within the primers or between two primers, and complementary sequence less than 3 bases at the 3'-end of the primers.

*Please search the specificity of the designed primers by NCBI BLAST to avoid non-specific amplification.

Troubleshooting

1. No or Low Yield of PCR Products

Primers	Optimize primer design
Annealing Temperature	Set gradient annealing temperature to find out the optimal one
Concentration of Primers	Appropriately improve the concentration of primers
Extension Time	Appropriately increase the extension time to 30 sec/kb-1 min/kb
Cycle Numbers	Increase cycle numbers to 35 - 40
Purity of Templates	Use high - purity templates
Template Input	Refer to the recommended reaction system and increase the input properly
Enzymes Input	Appropriately adjust the input of high-fidelity polymerase

2. Unspecific or Smear Bands in Electrophoresis

Primers	Optimize primer design
Annealing Temperature	Try to improve annealing temperature and set gradient annealing temperature to optimize
Concentration of Primers	Decrease the concentration of primers to final concentration as 0.2 μM
Extension Time	Appropriately decrease the extension time when blend bands longer than target bands appear
Cycle Numbers	Decrease cycle numbers to 25 - 30
PCR Programs	Use Two-Step PCR or Tough down PCR
Purity of Templates	Use high purity templates
Template Input	Modify or decrease templates input refering to the recommended reaction system
Template Input	Appropriately adjust or decrease the input of high - fidelity polymerase