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Lab & Production Materials

Data Sheet

Acculong DNA Polymerase

Product Number: D5121

Product Description

Acculong DNA Polymerase is a new generation superior enzyme based on Acculong DNA Polymerase for robust PCR with higher fidelity. The unique extension factor, specificity-promoting factors and plateau un-inhibitory factor newly added to Acculong greatly improve its long-fragment amplification ability, specificity, and PCR yield. Acculong is capable of amplifying long fragments such as 40 kb λDNA, 40 kb plasmid DNA, 20 kb genomic DNA and 10 kb cDNA. The amplification error rate of Acculong is 53-fold lower than that of conventional Taq and 6-fold lower than that of Pfu. In addition, Acculong 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. Acculong contains two monoclonal antibodies inhibiting the 5'→3' polymerase activity and 3'→5' exonuclease activity at room temperature, which enable Acculong to perform hot start PCR with great specificity. Amplification will generate blunt-ended products.

Components

Components	D5121-100U	D5121-500U
Acculong DNA Polymerase	100 µl	
2 x Buffer	2 x 1.25 ml	
dNTP Mix (10 mM each)	100 µl	5 x D5121-100U
10 x DNA Loading Buffer	12.5	

Storage

Store at -30 ~ -15°C and transport at ≤ 0°C

Applications

This product is suitable for PCR amplification using genomic DNA, cDNA, Plasmid DNA and crude samples as templates.

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°C with activated salmon sperm DNA as the template/primer.

Notes

1. Please use high quality DNA as templates.
2. Please ensure that the primers and templates do not contain uracil. And do not use dUTP.
3. If necessary, appropriately increase the amount of Acculong DNA Polymerase. For 50 µl reaction solution, the amount of Acculong DNA Polymerase should not exceed 2 U.
4. Acculong DNA Polymerase has strong proof-reading activity. If TA cloning needs to be performed, please perform purification before dA-tailing.
5. To prevent the degradation of primers due to the proof-reading activity of Acculong DNA Polymerase, please add the polymerase at last when preparing the reaction mixture.
6. Primer Design
   It is recommend that the last base at the 3' end of primer should be G or C.
   Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer.
   Avoid hairpin structures at the 3' end of the primer.
   Differences in the Tm value of the forward primer and the reverse primer should be no more than 1°C and the Tm value should be adjusted to 55°C to 65°C (Primer Premier 5 is recommended to calculate the Tm value).
   Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value.

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Control the GC content of the primer to be 40% - 60%.
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.
Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers and avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
Use the NCBI BLAST function to check the specificity of the primer to prevent non-specific amplification.

Experiment Process

For Conventional PCR

Recommended PCR System

Keep all components on ice during the experiment. Thaw, mix and briefly centrifuge each component before use. And put back to -20°C for storage.

Components	Volume
ddH_2O	up to 50 µl
2 x Buffer[a]	25 µl
dNTP Mix(10 mM each)	1 µl
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
Acculong DNA Polymerase Template	1 µl
DNA[b]	x µl

a) It contains Mg^{2+} with a final concentration of 2 mM.

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

Template Type	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)

Recommended PCR Program

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

a. For initial-denaturation, the recommended temperature is 95°C, and the recommended time is 30 sec for plasmid/virus DNA and 3 min for genomic DNA/cDNA.

b. Set the annealing temperature according to the Tm value of the primers. If the Tm value of the primers is higher than 72°C, the annealing step can be removed (two-step PCR). If necessary, annealing temperature can be further optimized through setting temperature gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature is helpful to improve amplification specificity.

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

For Long-fragment PCR

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

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

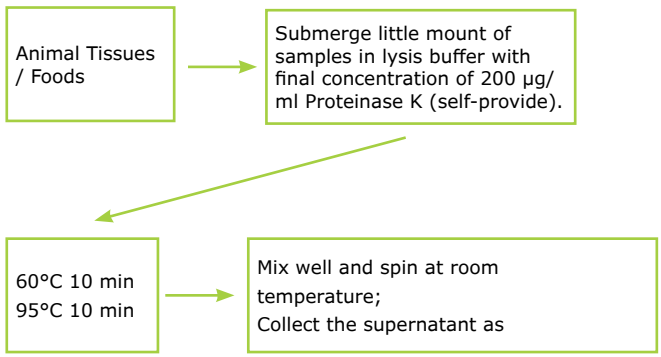
▲ It is recommended to use high-quality templates and long primers. Increasing the input amount of template DNA may be helpful to improve the products yield.

For PCR Using Crude Sample as Template

Acculong DNA Polymerase has 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 samples that have been successfully amplified with Master Mix are as follows:

Sample Type	Amplification Method	Template Recommendation (for a 50 µl PCR system)
Whole Blood	Direct PCR	1 -5 µl
Dry Serum Filter Paper	Direct PCR	1 - 2 mm^2 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
Mildew	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 mm^2 tissue
Mouse Tail	PCR with lysate	1 -5 µl lysate
Food	PCR with lysate	1 -5 µl lysate

▲ Lysate Prepration:



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

Examples

Amplification of Various Long Fragments

Taking human genomic DNA as templates, the target fragments of 0.6 kb, 1.0 kb, 2.6 kb, 3.0 kb, 4.0 kb, 5.1 kb, 6.2 kb, 7.1 kb, 8.5 kb, 10.6 kb, 17.8 kb, 20.3 kb, and 21.4 kb were amplified, respectively. The Tm value of all primers are approximately 60°C (calculated by Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

Components	Volume
ddH_2O	up to 50 μl
2 x Buffer	25 μl
dNTP Mix(10 mM each)	1 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Acculong DNA Polymerase	1 μl

Recommended PCR Program

Steps	Temperature	Time	Cycles
Initial-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	35
Annealing	60°C	15 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

Stable Amplification Ability of Crude Samples

1. Taking the human whole blood collected with EDTA blood collection tube as template, a target fragment of 1,295 bp was amplified with Acculong DNA Polymerase, a high fidelity DNA polymerase from company A, and a high fidelity DNA polymerase from company B, respectively. In addition, 2 × Acculong Master Mix was used to amplify longer fragments (3,276 bp and 8,513 bp). The Tm value of all primers are around 60°C (calculated by Primer Premier 5). The reaction system and program are as follows:

Components	Volume
ddH_2O	up to 50 μl
2 x Buffer	25 μl
dNTP Mix(10 mM each)	1 μl
Whole Blood*	x μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Acculong DNA Polymerase	1 μl

*The input amounts of the whole blood are 1 μl, 2 μl, 4 μl, respectively.

Recommended PCR Program

Steps	Temperature	Time	Cycles
Initial-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	35
Annealing	60/63/70°C	15 sec	
Extension*	72°C	30 sec/kb	
Final Extension	72°C	5 min	

* The annealing temperatures for 1.3 kb, 3.6 kb and 8.5 kb of target fragments are 60°C, 63°C, and 70°C, respectively.

2. Taking the tomato leaf, rice leaf, polished rice as templates, and the purified genomic DNA from rice leaf as positive control, target fragments of 1.3 kb were amplified with Acculong DNA Polymerase, a high fidelity DNA polymerase from company A, and a high fidelity DNA polymerase from company B, respectively. The Tm value of all primers are approximately 60°C (calculated by Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

Components	Volume
ddH_2O	up to 50 μl
2 x Buffer	25 μl
dNTP Mix(10 mM each)	1 μl
Plant Tissues*	x μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Acculong DNA Polymerase	1 μl

*The recommended diameter of the plant tissues is 0.3 - 3 mm.

Recommended PCR Program

Steps	Temperature	Time	Cycles
Initial-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	35
Annealing	60°C	15 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

3. Using the lysate of mouse tails as templates, a target fragment of 2.5 kb was amplified with Acculong DNA Polymerase, a high fidelity DNA polymerase from company A, and a high fidelity DNA polymerase from company B, respectively. The Tm value of all primers are approximately 60°C (calculated by Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

Components	Volume
ddH_2O	up to 50 μl
2 x Buffer	25 μl
dNTP Mix(10 mM each)	1 μl
Lysate of Mouse Tails	2 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Acculong DNA Polymerase	1 μl

Recommended PCR Program

Steps	Temperature	Time	Cycles
Initial-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	
Annealing	60°C	15 sec	35
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

Excellent Ability of Fragments with High GC Content

Acculong DNA Polymerase is capable of amplifying GC-rich fragments that conventional polymerase cannot amplify. Taking human genomic DNA as templates, target fragments of 654 bp, 900 bp, 800 bp, 1,200 bp, 1,400 bp, and 426 bp were amplified, respectively, The GC contents of all these amplicons are higher than 68%. High amplification efficiency is shown in the following figure. The Tm value of all primers are approximately 60°C (calculated by Primer Premier 5). Refer to 07-1 for reaction system preparation, and the PCR program is as follows:

Steps	Temperature	Time	Cycles
Initial-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	} 35
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

Reliable High Fidelity

The amplification fidelity of Acculong DNA Polymerase is 53-fold superior than that of Taq DNA Polymerase and 6-fold higher than that of Pfu DNA Polymerase.

FAQs & Troubleshooting

No amplification products or low amount of amplification products	
Primer	Optimize primer design
Annealing temperature	Set temperature gradient and find the optimal annealing temperature
Primer concentration	Increase the primer concentration appropriately
Extension time	Appropriately increase the extension time to 30 sec/kb - 1 min/kb
Cycles	Increase the cycles to 35 - 40
Template purity	Use templates with high purity
Enzyme amount	Appropriately increase the amount of the enzyme

Unspecific products or smear bands	
Primer	Optimize primer design
Annealing temperature	Set temperature gradient and find the optimal annealing temperature
Primer concentration	Decrease the final concentration of primer to 0.2 μM
Extension time	Appropriately decrease the extension time when larger unspecific fragments appear
Cycles	Decrease the cycles to 25 - 30
PCR program	Use two-step method or Touch Down PCR program
Template purity	Use templates with high purity
Enzyme amount	Appropriately decrease the amount of the enzyme

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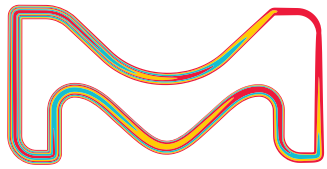

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