

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 × Buffer | 2 × 1.25 ml | |
| dNTP Mix (10 mM each) | 100 µl | 5 × D5121-100U |
| 10 × 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 T_m value of the forward primer and the reverse primer should be no more than 1°C and the T_m value should be adjusted to 55°C to 65°C (Primer Premier 5 is recommended to calculate the T_m value). Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer T_m value.

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 ₂ O | 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 Mg²⁺ 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 T_m value of the primers. If the T_m 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 |

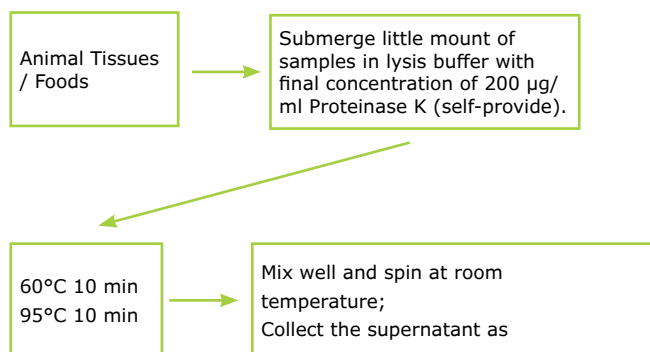
▲ It is recommended to use high-quality templates and long primers. Increasing the input amount of template DNA may be helpful to improve the products yield.

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 monoclonal or 1 µl suspension |
| Bacteria | Direct PCR | A monoclonal 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 Preparation:



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 T_m 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 ₂ O | 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 | } 35 |
| Denaturation | 95°C | 15 sec | |
| 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 x Acculong Master Mix was used to amplify longer fragments (3,276 bp and 8,513 bp). The T_m value of all primers are around 60°C (calculated by Primer Premier 5). The reaction system and program are as follows:

| Components | Volume |
|-------------------------|-------------|
| ddH ₂ O | 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 | } 35 |
| Denaturation | 95°C | 15 sec | |
| 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 T_m 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 ₂ O | 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 | } 35 |
| Denaturation | 95°C | 15 sec | |
| 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 T_m 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 ₂ O | 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 T_m 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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