TOOLS Ultra High Fidelity DNA Polymerase

Cat. no. TTC-PE05

Storage: Store at -20°C

Product Size: 100 U

Introduction

TOOLS Ultra High Fidelity DNA Polymerase is a new generation superior enzyme based on Ultra High Fidelity DNA Polymerase for robust PCR with relatively high fidelity. The unique extension factor, specificity-promoting factors, and plateau-inhibiting factor newly added to Ultra High Fidelity DNA Polymerase substantially improve its long-fragment amplification ability, specificity, and PCR yield. Ultra High Fidelity DNA Polymerase can amplify 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 Ultra High Fidelity DNA Polymerase is 53-fold lower than that of conventional Taq and 6-fold lower than that of Pfu. In addition, Ultra High Fidelity DNA Polymerase has a favorable resistance to PCR inhibitors and can be used for direct PCR amplification of bacterial, fungal, plant and animal tissue, and whole blood samples. Ultra High Fidelity DNA Polymerase contains two monoclonal antibodies inhibiting the 5′→3′ polymerase activity and 3′→5′ exonuclease activity at room temperature, which enable Ultra High Fidelity DNA Polymerase to perform highly specific hot start PCRs. The amplification generates blunt-ended products, which are compatible with the TOOLS UltraFast PCR cloning kit (TTC-CA15).

Product information

TOOLS Ultra High Fidelity DNA Polymerase (1 U/μL)	100 U
2× Ultra Buffer	2 × 1.25 mL
dNTP Mix (10 mM each)	100 μL
10× Loading buffer	1.25 mL

Unit definition

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

Important Notes

1. DO NOT use dUTP or any primers or templates that contain uracil.

2. Increase the input of Phanta Max Super-Fidelity DNA Polymerase according to the experiment requirements, but use no more than 2 U in a 50 μL reaction system.
3. Phanta Max Super-Fidelity DNA Polymerase has strong proofreading activity. Therefore, the PCR products must be purified before adding A-Tailing when TA cloning.
4. To prevent the strong proofreading activity of the Phanta Max Super-Fidelity DNA Polymerase degrading primers, the polymerase should be loaded last when creating the reaction system.

Protocol

Primer design

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 using a hairpin structure at the 3′-end of the primer.
4. Tm of the primers should be within 55–65 °C.
5. Additional sequence should not be included when calculating the Tm of the primers.
6. The GC content of the primers should be within 40%–60%.
7. The general distribution of A, G, T, and C in the primers should be uniform, and avoid using regions with rich GC and AT.
8. Keep the complementary sequence at fewer than 5 bases within the primers or between two primers, and fewer than 3 bases at the 3′-end of the primers.
9. Search the specificity of the designed primers by NCBI BLAST to avoid nonspecific amplification.

Recommended PCR reaction

Template	Optional
TOOLS Ultra High Fidelity DNA Polymerase (1 U/μL)	1 μL
dNTP Mix (10 mM each)	1 μL
Primer 1 (10 μM)	2 μL
Primer 2 (10 μM)	2 μL
2× Ultra Buffer[a]	25 μL
ddH_2O	Up to 50 μL

Note: [a] 2× Ultra Buffer contains Mg^{2+}. The final concentration of Mg^{2+} is 2 mM.

The 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)

PCR cycle setup：

95 °C[a]	30 s / 3 min	
95 °C	15 sec	25–35 cycles
56-72 °C[b]	15 sec	
72 °C[c]	30–60 sec/kb	
72 °C	5 min	

Note:

[a] For predenaturation, 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] For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72 °C, 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 on the annealing temperature. Increasing the annealing temperature in 3 °C increments is helpful to improve poor amplification specificity.

[c] A longer extension time is helpful to increase the amplification yield.

For long-fragment PCR

95°C[a]	3 min	
95°C	15 sec	5 cycles
74°C[b]	60 sec/kb	
95°C[c]	15 sec	5 cycles
72°C	60 sec/kb	
95°C[a]	15 sec	5 cycles
70°C	60 sec/kb	
95°C[b]	15 sec	25 cycles
68°C[c]	60 sec/kb	
68°C	5 min	

Note: Use high-quality templates and improve the template usage. Use long primers. When the

recommended PCR program does not perform optimally, try Touch Down Two-step PCR.

[a] For predenaturation, 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] For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72 °C, 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 on the annealing temperature. Increasing the annealing temperature in 3 °C increments is helpful to improve poor amplification specificity.

[c] A longer extension time is helpful to increase the amplification yield.

For PCR using crude materials as templates

TOOLS 2× Ultra Hi-Fi PCR Master Mix has a favorable resistance to PCR inhibitors and can be used for direct PCR amplification of bacterial, fungal, plant and animal tissue, and whole blood samples. Crude materials that have been effectively amplified with Phanta Max Master Mix are as follows.

Sample Type	Amplification Method	Template Recommendation[a]
Whole Blood	Direct PCR	1–5 µL
Filter Paper Dry Blood	Direct PCR	2 filter papers, 1–2 mm each
Cultured Cells	Direct PCR	Small amount of cells
Yeast	Direct PCR	A monoclone or 1 µL suspension
Bacteria	Direct PCR	A monoclone or 1 µL suspension
Mold	Direct PCR	Small amount of sample
Sperm	Direct PCR	Small amount of sample
Plankton	Direct PCR	Small amount of sample
Plant Tissue	Direct PCR	2 tissues, 1–2 mm each
Mouse Tail	PCR with lysate	1–5 µL of lysate[b]
Food	PCR with lysate	1–5 µL of lysate[b]

Note:

[a] For a 50-µL PCR system

[b] Lysate preparation: Submerge a small amount of the sample in lysis buffer with a final concentration of 200 µg/mL Proteinase K (self-provide) at 60 °C for 10 min and then 95 °C for 10 min. Mix well and spin at room temperature; collect the supernatant as lysate. Lysis buffer: 20 mM Tris-HCl, 100 mM EDTA, 0.1% SDS, pH 8.0

Application examples

The following is suitable for amplification of fragments with various samples.

1. By using human genomic DNA as templates, the target fragments of 0.6, 1.0, 2.6, 3.0, 4.0, 5.1, 6.2, 7.1, 8.5, 10.6, 17.8, 20.3, and 21.4 kb can be amplified. The Tm of all primers is approximately 60 °C (calculated using Primer Premier 5).

The reaction system and program:

Human Genomic DNA (100 ng/μL)	2 μL
TOOLS Ultra High Fidelity DNA Polymerase (1 U/μL)	1 μL
dNTP Mix (10 mM each)	1 μL
Primer 1 (10 μM)	2 μL
Primer 2 (10 μM)	2 μL
2× Ultra Buffer	25 μL
ddH_2O	Up to 50 μL

Recommended PCR reaction

95°C[a]	3 min	
95°C	15 sec	35 cycles
60°C[b]	15 sec	
72°C[c]	30 sec/kb	
72°C	5 min	

Note:

[a] For predenaturation, 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] For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72 °C, 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 on the annealing temperature. Increasing the annealing temperature in 3 °C increments is helpful to improve poor amplification specificity.

[c] A longer extension time is helpful to increase the amplification yield.

2. By using a human whole blood sample as a template, a target fragment of 1,295 bp can be amplified with TOOLS 2× Ultra Hi-Fi PCR Master Mix. The Tm of all primers is approximately 60 °C (calculated using Primer Premier 5).

The reaction system and program:

Whole blood	1–4 μL

TOOLS Ultra High Fidelity DNA Polymerase (1 U/μL)	1 μL
dNTP Mix (10 mM each)	1 μL
Primer 1 (10 μM)	2 μL
Primer 2 (10 μM)	2 μL
2× Ultra Buffer	25 μL
ddH_2O	Up to 50 μL

Recommended PCR reaction

95°C[a]	3 min	
95°C	15 sec	35 cycles
60/63/70°C[b]	15 sec	
72°C[c]	30 sec/kb	
72°C	5 min	

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

[a] For predenaturation, 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] For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72 °C, 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 on the annealing temperature. Increasing the annealing temperature in 3 °C increments is helpful to improve poor amplification specificity.

[c] A longer extension time is helpful to increase the amplification yield.

3. By using a tomato leaf, a rice leaf, and polished rice as templates and the purified genomic DNA from the rice leaf as positive control, target fragments of 1.3 kb can be amplified with TOOLS 2× Ultra Hi-Fi PCR Master Mix. The Tm of all primers is approximately 60 °C (calculated using Primer Premier 5).

The reaction system and program:

Plant tissues*	X μL
TOOLS Ultra High Fidelity DNA Polymerase (1 U/μL)	1 μL
dNTP Mix (10 mM each)	1 μL
Primer 1 (10 μM)	2 μL
Primer 2 (10 μM)	2 μL
2× Ultra Buffer	25 μL
ddH_2O	Up to 50 μL

Note: *The recommended diameter of the plant tissues is 0.3–3 mm.

Recommended PCR reaction

95°C[a]	3 min	
95°C	15 sec	35 cycles
60°C[b]	15 sec	
72°C[c]	30 sec/kb	
72°C	5 min	

Note:

[a] For predenaturation, 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] For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72 °C, 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 on the annealing temperature. Increasing the annealing temperature in 3 °C increments is helpful to improve poor amplification specificity.

[c] A longer extension time is helpful to increase the amplification yield.

4. By using the lysate of mouse tails as a template, a target fragment of 2.5 kb can be amplified with TOOLS 2× Ultra Hi-Fi PCR Master Mix. The Tm of all primers is approximately 60 °C (calculated using Primer Premier 5).

The reaction system and program:

Lysate of Mouse Tails	2 μL
TOOLS Ultra High Fidelity DNA Polymerase (1 U/μL)	1 μL
dNTP Mix (10 mM each)	1 μL
Primer 1 (10 μM)	2 μL
Primer 2 (10 μM)	2 μL
2× Ultra Buffer	25 μL
ddH_2O	Up to 50 μL

Recommended PCR program

95°C[a]	3 min	
95°C	15 sec	35 cycles
60°C[b]	15 sec	
72°C[c]	30 sec/kb	

72°C	7 min

Note:

[a] For predenaturation, 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] For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72 °C, 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 on the annealing temperature. Increasing the annealing temperature in 3 °C increments is helpful to improve poor amplification specificity.

[c] A longer extension time is helpful to increase the amplification yield.

5. Amplification of fragments with high GC content

When using human genomic DNA as templates, the GC contents of all the amplicons are expected to be higher than 68%. The Tm of all primers is approximately 60 °C (calculated using Primer Premier 5).

The reaction system and program:

TOOLS Ultra High Fidelity DNA Polymerase (1 U/μL)	1μL
dNTP Mix (10 mM each)	1 μL
Primer 1 (10 μM)	2 μL
Primer 2 (10 μM)	2 μL
2× Ultra Buffer	25 μL
ddH_2O	Up to 50 μL

Note: *The recommended diameter of the plant tissues is 0.3–3 mm.

Recommended PCR program

95°C[a]	3 min	
95°C	15 sec	35 cycles
72°C[c]	45 sec/kb	
72°C	5 min	

Note:

[a] For predenaturation, 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] For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72 °C, 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 on the annealing temperature. Increasing the annealing temperature in 3 °C increments is helpful to improve poor amplification specificity.

[c] A longer extension time is helpful to increase the amplification yield.

Troubleshooting

No or low yield of PCR products

Primers	Optimize the primer design
Annealing Temperature	Set a gradient annealing temperature to identify the optimal temperature
Primer Concentration	Appropriately increase the concentration of primers
Extension Time	Appropriately increase the extension time to 0.5–1 min/kb
Cycle Number	Increase cycle number to 35–40
Purity of Templates	Use high-purity templates
Template Input	Refer to the recommended reaction system and increase the input as appropriate

Unspecific or smear bands in electrophoresis

Primers	Optimize the primer design
Annealing Temperature	Increase the annealing temperature by setting a gradient annealing temperature
Primer Concentration	Reduce the concentration of primers to a final concentration of 0.2 μM
Extension Time	Shorten the extension time when blend bands are longer than target bands
Cycle Number	Reduce cycle number to 25–30
PCR Programs	Use Touch Down Two-step PCR
Purity of Templates	Use high-purity templates
Template Input	Modify or reduce the template input according to the recommended reaction system

This product is for research only, not for diagnostic and clinical use.