Phanta Max Super-Fidelity DNA Polymerase

P505

Version 23.1

Product Description

Phanta Max Super-Fidelity DNA Polymerase is an upgraded version of Phanta Super-Fidelity DNA Polymerase. Compared with the previous generation, Phanta Max has added the unique elongation factor, specificity-enhancing factor and plateau phase anti-inhibitor factor, which greatly improves the long-fragment amplification ability, amplification specificity and amplification yield. Phanta Max can efficiently amplify up to 40 kb simple templates (e.g. λDNA, plasmids), 20 kb complex templates (e.g. genomic DNA) and 10 kb cDNA. The amplification error rate of Phanta Max is 128-fold lower than that of conventional *Taq* DNA Polymerase. In addition, Phanta Max has a good resistance to PCR inhibitors and can be used for direct PCR amplification of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Phanta Max contains two monoclonal antibodies inhibiting the 5'→3' polymerase activity and 3'→5' exonuclease activity at room temperature, which enable it to perform hot start PCR with great specificity. Amplification products are blunt-ended, which are compatible with ClonExpress kits (Vazyme #C112/C113/C116) and TOPO cloning kit (Vazyme #C603).

Components

Components	P505-d1 (100 U)	P505-d2 (500 U)	P505-d3 (1,000 U)
Phanta Max Super-Fidelity DNA Polymerase	100 μl	5 × P505-d1	10 × P505-d1
2 × Phanta Max Buffer	2 × 1.25 ml		
dNTP Mix (10 mM each)	100 μl		
10 × DNA Loading buffer	1.25 ml		

Storage

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

Applications

It is applicable for amplification reaction of genomic DNA, cDNA, Plasmid DNA and crude samples as templates.

Notes

For research use only. Not for use in diagnostic procedures.

1. Please use high-quality templates.
2. Please do not use dUTP. Also, please ensure that the primers and templates are uracil-free.
3. If necessary, appropriately increase the amount of Phanta Max Super-Fidelity DNA Polymerase. For 50 μl reaction system, the amount of Phanta Max Super-Fidelity DNA Polymerase should not exceed 2 U.
4. Phanta Max Super-Fidelity DNA Polymerase has the strong proofreading activity. If TA cloning needs to be performed, it is recommended to purify the DNA before adding the adenine.
5. To prevent the degradation of primers due to the proofreading activity of Phanta Max Super-Fidelity DNA Polymerase, please add the polymerase at last when preparing the reaction mixture.
6. Primer Design Guidance
   a. It is recommended that the last base at the 3' end of the primer should be G or C.
   b. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer.
   c. Avoid hairpin structures at the 3' end of the primer.
   d. 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 ~ 65°C (Primer Premier 5 is recommended to calculate the Tm value).
   e. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value.
   f. It is recommended that the GC content of the primer to be 40% - 60%.
   g. 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.
   h. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers. Avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
   i. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

Experiment Process

Reaction System

Keep all components on ice during the experiment. Thaw, mix, and briefly centrifuge each fraction before use. After use, please return it to -20°C in time for storage.

Components	Volume
ddH_2O	up to 50 μl
2 × Phanta Max Buffer[a]	25 μl
dNTP Mix (10 mM each)	1 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Phanta Max Super-Fidelity DNA Polymerase	1 μl
Template 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 Types	Amount
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)

Reaction Program

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

a. Please set the annealing temperature according to the Tm value of the primers. When 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.

b. Properly extending the extension time can improve the amplification yield.

FAQ & Troubleshooting

◇ No amplification products or low yield

① Primer: Optimize primer design.

② Annealing temperature: Set temperature gradient and find the optimal annealing temperature.

③ Primer concentration: Increase the concentration of primers properly.

④ Extension time: Increase the extension time to 30 sec/kb - 1 min/kb properly.

⑤ Cycles: Increase the number of cycles to 36 - 40 cycles.

⑥ Template purity: Use templates with high purity.

⑦ Template amount: Adjust the template amount according to the recommended amount and increase it properly.

◇ Nonspecific products or smeared bands

① Primer: Optimize primer design.

② Annealing temperature: Try to increase the annealing temperature and set temperature gradient.

③ Primer concentration: Decrease the concentration of primers properly.

④ Cycles: Decrease the number of cycles to 25 - 30 cycles.

⑤ PCR program: Use Two-Step method or Touchdown PCR program.

⑥ Template purity: Use templates with high purity.

⑦ Template amount: Adjust the template amount according to the recommended amount and decrease it properly.