Phanta Super-Fidelity DNA Polymerase

P501

Version 23.1

Product Description

Phanta Super-Fidelity DNA Polymerase is a new generation of super-fidelity DNA polymerase modified based on Pfu DNA Polymerase. With high amplification efficiency and board template applicability, it is suitable for almost all amplification reaction. After the genetic engineering of Pfu DNA Polymerase, the performance of Phanta Super-Fidelity DNA Polymerase has been greatly improved, and it can complete the reaction accurately and quickly even with complex templates. Its error rate 52-fold lower than that of Taq DNA Polymerase, and 6-fold lower than that of Pfu DNA Polymerase. Its amplification speed can reach 15 - 30 sec/kb. Phanta Super-Fidelity DNA Polymerase is the preferred enzyme for high-fidelity PCR because of its high fidelity and excellent amplification efficiency. This product has 5'→3' polymerase activity and 3'→5' exonuclease activity. Amplification products are blunt-ended, which are compatible with ClonExpress and TOPO cloning kits (Vazyme #C112/C113/C116/C603).

Components

Components	P501-d1 (100 U)	P501-d2 (500 U)	P501-d3 (1,000 U)
5 × SF Buffer (with 10 mM $MgSO_4$)	1.25 ml		
25 mM $MgSO_4$	1 ml		
dNTP Mix (10 mM each)	100 µl		
Phanta Super-Fidelity DNA Polymerase	100 µl	5 × P501-d1	10 × P501-d1
5 × PCR Enhancer	500 µl		
DMSO	100 µl		
10 × DNA Loading buffer	1.25 ml		

Storage

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

▲ Avoid repeated freezing and thawing.

Applications

It is applicable for amplification reaction of 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

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

1. Please use high-quality 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 Phanta Super-Fidelity DNA Polymerase. For 50 µl reaction system, the amount of Phanta Super-Fidelity DNA Polymerase should not exceed 2 U.
4. Phanta 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 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
5 × SF Buffer (with 10 mM $MgSO_4$)	10 µl
dNTP Mix (10 mM each)	1 µl
25 mM $MgSO_4$[a]	optional
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
Phanta Super-Fidelity DNA Polymerase	1 µl
Template DNA[b]	x µl

a. For most reactions, the optimal final concentration of Mg^{2+} is 1.5 - 2 mM. The system already contains a final concentration of 2 mM Mg^{2+}. If necessary, the 25 mM $MgSO_4$ provided in the kit can be used to search for the optimal concentration of Mg^{2+} at intervals of 0.2 - 0.5 mM.

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

	<1 kb	1 - 10 kb	>10 kb
Genomic DNA	50 - 250 ng	100 - 300 ng	150 - 400 ng
Plasmid or Viral DNA	10 pg - 20 ng	10 pg - 20 ng	1 - 30 ng
cDNA	1 - 5 µl (≤1/10 of the total volume of PCR system)		

Reaction Program

Steps	Temperature	Time	Cycles
Initial Denaturation[a]	95°C	30 sec - 3 min	
Denaturation[b]	95°C	5 - 10 sec	
Annealing[c]	60°C	10 - 30 sec	25 - 35
Extension	72°C	15 - 30 sec/kb	
Final Extension	72°C	5 - 10 min	

a. For initial denaturation, the recommended temperature is 95°C. If the amplicon exceeds 10 kb, the temperature of initial denaturation can be reduced to 92°C, and the time should not exceed 2 min.

b. For denaturation, the recommended condition is 5 - 10 sec at 95°C. If the amplicon exceeds 10 kb, the temperature of denaturation can be reduced to 92°C, and the time should be extended to 15 sec.

c. In general, the annealing temperature can be set within the range of the primer Tm value ±3°C. If necessary, the annealing temperature can be further optimized by setting a temperature gradient. If the annealing time is too long, the amplification product may appear as smeared bands. Therefore, it is recommended to set the annealing time to 10 sec. For some difficult templates, the annealing time can be adjusted between 10 - 30 sec.

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.