PowerPol 2X PCR Mix

Cat.No. : RK20718

Product Components

Components	Component Number	Size-1 1 mL	Size-2 5 mL	Size-3 100 mL
PowerPol 2X PCR Mix	RM20387	1 mL	1 mL × 5	1 mL × 100

Product Description

PowerPol 2X PCR Mix is an optimized premix containing DNA polymerase, dNTPs, $MgCl_2$, KCI and other stabilizers. It only needs to add primers and templates to perform amplification.

This product can use complex genomic DNA as a template to amplify a target fragment of 5 kb in length or a simple template such as lambda DNA to amplify a target fragment of 10 kb in length. It is suitable for conventional PCR reaction, vector construction, and other applications.

5′-3′exonuclease activity: No

3′-5′exonuclease activity: Yes

Fidelity: 6X *Taq*

Product End: Blunt end

Storage

-20℃

Operation Description

Standard Protocol

1. It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 98℃.

Recommended Reaction

Component	25 μL Reaction	50 μL Reaction	Final Concentration
PowerPol 2X PCR Mix	12.5 μL	25 μL	1X
Forward Primer (10 μM)	0.5 μL	1 μL	0.2 μM
Reverse Primer (10 μM)	0.5 μL	1 μL	0.2 μM
DNA Template*	Variable	Variable	<300 ng
Nuclease-free Water	to 25 μL	to 50 μL	N/A

* Note: The optimal reaction concentration varies with different DNA templates. Please refer to the basic principles of PCR below.

Recommended PCR Program:

Step	Temp	Time	Cycles
Initial Denaturation	98℃	45 s*	1
Denaturation	98℃	10 s	30
Annealing	55-65℃	30 s	
Extension	72℃	20-30 s/kb	
Final Extension	72℃	5 min	1
Hold	4-12℃	-	1

*Note: In the case of high complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation.

PCR Principles

1. Template

High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed Below (For a 50 μL reaction):

DNA	Input Amount
Plants, animals and human gDNA	10 ng-100 ng
E.coli, lambda gDNA	500 pg-200 ng
Plasmid DNA	1 pg-10 ng

Note: If the DNA template is obtained from a cDNA synthesis reaction, the template volume should be less than 10% of the total reaction volume. If long fragments are amplified, the amount of template input should be increased appropriately.

2. Primers

Oligonucleotide primers are typically 20-40 nucleotides in length with a GC content of 40-60%. Primers can be designed and analyzed using software such as Primer 3. The final concentration of each primer in the PCR reaction system should be in the range of 0.1-1 μM.

3. Denaturation

98℃ pre-denaturation for 45 s can fully denature most DNA templates. In the case of high complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation. Generally, the recommended denaturation condition for low-complexity DNA templates is 98℃, 5-10 s.

4. Annealing

The annealing temperature of PowerPol 2X PCR Mix is usually higher than other PCR polymerases. Generally, primers longer than 20 nt are annealed at (lower primer Tm+3)℃ for 10-30 s; when the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm. When using new primer pairs for amplification, it is recommended to determine the optimal annealing temperature through temperature gradient testing. In a two-step amplification protocol, the annealing temperature can be set the same as the extension temperature.

5. Extention

The recommended extension temperature is 72℃. The extension time depends on the length and complexity of the amplicon. For the low-complexity amplicons (plasmid DNA), the extension condition is 10 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 20-30 s/kb. In some cases, the extension time for cDNA templates should be less than 1 min/kb.

6. Cycles

To obtain enough yield of PCR products, 25-35 cyclesare recommended.