2× PCR Super Mix (without Dye)

Cat. No.: HYA449

Product Description

2× PCR Super Mix (without Dye) is an optimized premix contains DNA polymerase, dNTPs, $MgCl_2$, KCl, and other stabilizing agents. Simply add the template DNA and primers to perform PCR amplification. The amplified products have blunt ends.

This product can amplify target fragments of up to 5 kb from complex genomic DNA templates or up to 10 kb from simple templates such as lambda DNA. With a certain degree of amplification fidelity, it is suitable for a variety of applications, including colony PCR screening, genetic marker genotyping, plant transgene detection, and vector construction.

Components

Components	Specification
2× PCR Super Mix (without Dye)	1 mL × 5

Storage

Store at -20°C.

Fidelity

6× Taq.

Instructions

1. It is recommended to assemble all reaction components on ice, then promptly transfer the reaction mixture to a PCR thermal cycler preheated to 98°C.
2. PCR reaction:

Component	25 μL Reaction	50 μL Reaction	Final Concentration
2× PCR Super Mix (without Dye)	12.5 μL	25 μL	1×
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	Up to 25 μL	Up to 50 μL	/

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

3. PCR program:

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

*Note: For complex templates, such as GC-rich sequences, the initial denaturation time may be extended to 3 min to ensure complete denaturation.

PCR Principles

1. Template

Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 μL reaction are as follows:

DNA	Input Amount
Plants, animals and human gDNA	10 ng-100 ng
E.coli, lambda gDNA	100 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 2× PCR Super Mix (without Dye) 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, ananneal ing 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 cycles are recommended.