Taq 2X PCR Mix with Dye V2

Cat.No. : RM20391

Product Components

Components	Size 5 mL
Taq 2X PCR Mix with Dye V2	1 mL × 5

Product Description

Taq 2X PCR Mix with Dye V2 is an optimized PCR premixed solution containing *Taq* DNA Polymerase, dNTPs, $MgCl_2$, KCl, Bromophenol blue tracking dye, and other stabilizers. User only need to add template and primer to complete experiment. The amplified products were directly used for agarose gel electrophoresis.

It is ideally suited to routine PCR applications on various templates including pure DNA solutions, bacterial colony/culture, and cDNA products. It can amplify 5 kb DNA from different sources genomic DNA. It is applicable to PCR reaction, colony PCR identification, rough sample amplification.

5´-3´exonuclease activity: Yes

3´-5´exonuclease activity: No

Product End: Single-base 3´ Overhangs

Storage

-20℃

Operation Description

Standard Protocol

1. We recommend assembling all reaction components on ice and quickly transferring the reaction system to the PCR instrument preheated at 94℃.

Recommended Reaction

Component	25 μL Reaction	50 μL Reaction	Final Concentration
Taq 2x PCR Mix with Dye V2	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	94℃	3 min*	1
Denaturation	98℃	5-10 s	30
Annealing	55-60℃	20-30 s	
Extension	65℃	1 kb/min	
Final Extension	65-68℃	5 min	1
Hold	4-12℃	-	1

*Note: The recommended pre-denaturation time for colony PCR is 2-5 min.

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 generally 20-40 nucleotides in length and ideally have a GC content of 40-60%. Computer programs such as Primer3 can be used to design or analyze primers. The final concentration of each primer in a reaction may be 0.05-1 μM, typically 0.1-0.5 μM.

3. Mg^{2+} and Additives

In *Taq* 2X PCR Mix with Dye V2, the concentration of Mg^{2+} should be 4 mM, dNTP should be 300 μM. Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO or formamide.

4. Denaturation

An initial denaturation of 3 minutes at 94℃ is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 5 minutes to fully denature the template. With colony PCR, an initial 5 minutes denaturation at 94℃ is recommended to fully decompose the bacteria.During cycling a 10 seconds denaturation at 98℃ is recommended.

5. Extention

The recommended extension temperature is 65℃. The extension time is related to the length of the amplified fragment. Calculate the extension time at the speed of 1 kb/min. A final extension of 5 minutes at 65℃ is recommended.

6. Cycles

Generally, 25-35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.