2× PCR Mix (with Dye)

Cat. No.: HYA442

Product Description

2× PCR Mix (with Dye) is an optimized blue PCR premix containing Taq DNA polymerase, dNTPs, $MgCl_2$, KCl, and other stabilizers. Simply add the DNA template and primers to perform PCR amplification. Taq DNA polymerase possesses 5′→3′ polymerase activity and 5′→3′ exonuclease activity, but lacks 3′→5′ exonuclease activity. The resulting PCR products carry a single 3′-dA overhang, making them suitable for ligation into vectors with dT or dU overhangs.

This product is suitable for routine PCR amplification using purified DNA, bacterial colonies or cultures, crude extracts, cDNA, and other templates. It can amplify target fragments up to 5 kb from genomic DNA of various origins. Applications include routine PCR, colony PCR screening, and amplification from crude samples.

Components

Components	Specification
2× PCR Mix (with Dye)	1 mL × 5

Storage

Store at -20°C.

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 94°C.
2. PCR reaction:

Component	25 μL Reaction	50 μL Reaction	Final Concentration
2× PCR Mix (with 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	94℃	3 min*	1
Denaturation	98℃	5-10 s	
Annealing	55-60℃	20-30 s	30
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 2× PCR Mix (with Dye), the concentration of Mg^{2+} should be 4 mM, dNTPs 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 cyclinga10seconds 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.