2× Hotstart PCR Mix (without Dye)

Cat. No.:HYA461

Product Description

2× Hotstart PCR Mix (without Dye) is an ultra-efficient premix suitable for fast PCR. It contains Hot Start Taq DNA Polymerase, extension and amplification enhancers, and an optimized buffer system, achieving a maximum extension rate of 1 s/kb for fragments up to 1 kb, significantly reducing PCR run time. The antibody-blocked polymerase is completely inhibited at room temperature, preventing non-specific amplification and primer-dimer formation during setup.

It is suitable for amplifying fragments up to 5 kb from genomic DNA templates, or up to 10 kb from plasmid and λ DNA templates. To perform amplification, simply add primers and template to minimize pipetting steps and increase throughput. The stabilizer in the mix ensures consistent activity even after repeated freeze-thaw cycles.

Components

Components	Specification
2× Hotstart PCR Mix (without 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 95°C.
2. PCR reaction:

Component	25 μL Reaction	50 μL Reaction	Final Concentration
2× Hotstart PCR Mix (without Dye)	12.5 μL	25 μL	1×
Forward Primer (10 μM)	1 μL	2 μL	0.4 μM
Reverse Primer (10 μM)	1 μL	2 μL	0.4 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. The recommended DNA template amounts for a 50 μL reaction areas follows. For long fragment amplification, the amount of template input can be appropriately increased:

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
cDNA	1–5 μL (not exceeding 10% of the total PCR reaction volume)

3. PCR program:

Step	Temp	Time	Cycles
Initial Denaturation	95℃	3 min	1
Denaturation	98℃	10 s	
Annealing	60℃*	30 s	28-35
Extension	65℃	1-10 s/kb**	
Final Extension	65℃	10 min	1
Hold	4-12℃	-	1

*Note: Set the annealing temperature 3–5°C below the primer Tm. For complex templates, adjust the annealing temperature and extend the extension time to achieve efficient amplification.

**Note: Set the extension time according to target fragment length as follows:

PCR type	Amplicon length	Recommended extension time
Singleplex PCR	<2 kb	1 s/kb
	<5 kb	5 s/kb
	≤10 kb	10 s/kb

This product supports 9-plex amplification of fragments ≤10 kb. For multiplex amplification, a recommended extension time of 10–30 s/kb is suggested.

PCR Principles

1. Primers

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer5 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.

2. Mg^{2+} and Additives

In 2× Hotstart PCR Mix (without Dye), the concentration of Mg^{2+} should be 6 mM, dNTP should be 800 μM. Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO or formamide.

3. Denaturation

An initial denaturation of 3 minutes at 95℃ is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 5 minutes is recommended to fully denature the template.

4. Extension

The recommended extension temperature is 65°C. The extension time is related to the length of the amplified fragment. Calculate the extension time using an extension rate of 1-10 s/kb. A final extension of 10 minutes at 65℃ is recommended.

5. Cycles

Generally, 28–35 cycles yield sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.