2× HiFi PCR Mix (with Dye)

Cat. No.:HYA446

Product Description

2× HiFi PCR Mix (with Dye) contains HiFi DNA polymerase, dNTP, and an optimized buffer system. Only primers and templates needed to be added for amplification, minimizing pipetting steps, significantly controlling cross-contamination among samples, and improving detection throughput and reproducibility of results. The folmulation includes protective agents that ensure stable enzymatic activity even after repeated freeze-thaw cycles. 2× HiFi PCR Mix (with Dye) contains loading buffer, PCR products can be directly loaded for electrophoresis after the reaction.

5′-3′exonuclease activity: No

3′-5′exonuclease activity: Yes

Product End: Blunt end

Components

Components	Size-1	Size-2
2× HiFi PCR Mix (with Dye)	1 mL	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× HiFi 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. 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	95℃	15 s	
Annealing	60℃*	30 s	25-35
Extension	72℃	10 s/kb**	
Final Extension	72℃	5 min	1
Hold	4-12℃	-	1

*Note: In general, the annealing temperature can be set to 60℃ by default, and for primers longer than 20 nt, annealing temperature should be set to (lower primer Tm+3)℃, and for primers shorter than 20 nt, the annealing temperature should be equivalent to the lower primer Tm.

**Note: Properly extending the extension time can improve the amplification yield. Generally, it can be extended at a speed of 10 s/kb. For more complex templates or fragments with a length of more than 10 kb, the extension time can be appropriately extended to 15 s/kb.

FAQs

1. Low or no amplification yields

a. Problems with PCR primers: setting up a positive control reaction.

b. The annealing temperature is not suitable: adjust the annealing temperature and carry out gradient optimization experiments.

c. Low primer concentration or insufficient cycle number in PCR system: increase the amount of primer appropriately, or increase the number of amplification cycles.

d. Insufficient extension time: extend the extension time to 15 s/kb. e. Effect of inhibitors: use a high-purity template, or amplify after template dilution.

2. Generate non-specific amplified bands

a. The primer concentration or DNA template concentration in the PCR system is too high: reduce the amount of primers and template appropriately.

b. The annealing temperature is too low or the number of cycles is too high: gradient optimization experiments can be carried out by increasing the annealing temperature or decreasing the number of cycles.

c. PCR primer mismatch: redesign PCR primers.