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2× High-Fidelity PCR Master Mix

1 Components

Components	HY-K0533-1 mL	HY-K0533-5 mL
2× High-Fidelity PCR Master Mix	1 mL	1 mL ×5

2 Introduction

MCE 2× High-Fidelity PCR Master Mix is a new high-fidelity PCR amplification premix. The product contains a new type of modified high-fidelity DNA Polymerase with higher 5′→3′polymerase activity and higher 3′→5′exonuclease activity. The additional hot-start factor can be activated to improve specificity and stability of PCR amplification by minimizing the non-specific amplification and enzyme activity loss. The product also contains a unique Extension-Enhancement factor, making the extending speed and the amplification efficient significantly improved. It can effectively amplify the fragments up to 10 kb, and the amplification speed can reach 15 sec/kb.

MCE 2× High-Fidelity PCR Master Mix is a ready-to-use premix. With the primers and template added, the optimized system will provide sensitive and reliable DNA synthesis. It saves preparation time, reduces the risk of contamination from multiple pipetting steps, and provides consistent reaction-to-reaction performance. This product is no DNA loading buffer, the DNA loading buffer should be added to the PCR product when run on the agarose gel. The PCR product is blunt-ended.

3 Protocol

1. Prepare PCR reaction mixture

To obtain reliable PCR reaction results, the suggested template amount is 50 ng to 200 ng for genomic DNA, 10 pg to 20 ng for plasmid or viral DNA, 1 μL to 5μL for cDNA (≤1/10 of the total volume of PCR system).

Please prepare the PCR reaction solution according to the list below (all reagents should be placed on ice).

Reagent	Volume
2× High-Fidelity PCR Master Mix	25 μL
PCR Forward Primer (10 μM)	2.5 μL
PCR Reverse Primer (10 μM)	2.5 μL
DNA	x μL
ddH_2O	to 50 μL

Notes:

a. 0.5 μM of primer final concentration is applicable for most cases. The concentration can be adjusted within 0.2~1.0 μM when amplification efficiency is not satisfactory.

b. The product provides 1.5 mM Mg^{2+} in 1× concentration and 1 U/50 μL polymerases.

2. Common cycling parameters for PCR

a. Perform PCR using optimized cycling conditions. Provided below is a standard two-step program and three-step program.

Step	Temp.	Time	Cycles
Initial denaturing	98°C	3 min	1
Denaturation	98°C	10 sec	30-35
Extension	68°C	30 sec/kb	
Final extension	72°C	5 min	1

Step	Temp.	Time	Cycles
Initial denaturing	98°C	3 min	1
Denaturation	98°C	10 sec	30-35
Annealing	60°C	20 sec	
Extension	72°C	30 sec/kb	
Final extension	72°C	5 min	1

b. For Long-fragment PCR, if the recommended program is failure to work, the following PCR program may be helpful.

Step	Temp.	Time	Cycles
Initial denaturing	98°C	3 min	1
Denaturation	98°C	10 sec	15 (1°C reduction per cycle)
Gradient annealing	70-55°C	20 sec	
Extension	72°C	30 sec/kb	
Denaturation	98°C	10 sec	20
Annealing	55°C	20 sec	
Extension	72°C	30 sec/kb	
Final extension	72°C	5 min	1

Notes:

a. For initial denaturing program setting, the temperature is recommended to be 98°C, and the time is 3 min. If the GC content of the template is high, the time can be extended by 2-7 min.

b. For annealing program setting, the temperature is recommended to be 60°C, and the time is 20 sec.

c. For extension program setting, the temperature is recommended to be 72°C, and the time is 30 sec/kb. If the template is more complicated, the time can be extended to 60 sec/kb.

d. Please store the PCR product at -20°C to prevent the enzyme from degrading the products. The PCR product is blunt-ended.

4 Storage Conditions

Store at -20°C for 2 years. 4°C for short-term storage (up to 3 months).

Avoid repetitive freeze-thaw cycles while using.

5 Precautions

a. Gently invert the tube upside down several times before use. Brief centrifugation prior to use is recommended.

b. It is recommended to set up reaction systems on ice.

c. This product is for R&D use only, not for drug, household, or other uses.

d. For your safety and health, please wear a lab coat and gloves while handling.