

## Hi-Fi PCR Master Mix (2X)

**Catalogue No.:** K090

**Size:** 1mL/1ml\*5

### Storage:

The kit should be stored at -20°C for at least one year. Avoid repeated freezing and thawing. For frequent use, appropriate amount can be stored at 4°C for at least 3 days.

### Introduction:

Hi-Fi PCR Master Mix (2X) uses high-fidelity DNA Polymerase, amplification rate can reach 15s/kb, contains 2X Hi-Fi DNA Polymerase, 2X PCR Buffer(with Mg<sup>2+</sup>), 2X dNTP, only need to add the right amount of primers, templates and water to perform high-fidelity PCR amplification. The PCR operation is greatly simplified, the operation is faster, the pollution that may be caused during the PCR operation is reduced, and the repeatability of PCR is better.

The product can perform a variety of conventional PCR amplification and is particularly suitable for high-fidelity and rapid qualitative and semi-quantitative PCR amplification, as well as for the amplification of long fragments of DNA (up to 12kb in length).

The amplification speed is extremely fast. When the amplification of DNA fragments smaller than 6kb, the extension of 1kb takes only 15 seconds. Normal DNA polymerase elongation of 1kb usually takes 1-2 minutes. Its error probability is 52 times lower than that of Taq enzyme and 6 times lower than that of pfu enzyme.

This product has high stability and has no significant effect on PCR amplification after repeated freezing and thawing for 15 times.

For 50 microliter PCR reaction system, the 1mL size is enough for 40 samples; For 20 microliters of PCR reaction system, enough for 100 samples.

### Assay Procedure:

#### 一. PCR reaction system was set up:

1. Melt and mix the solutions required for PCR reaction. Place the Hi-Fi PCR Master Mix (2X) on an ice bath or in an ice box.
2. Refer to the table below to set up the PCR reaction system under ice bath conditions.

reagents	final concentration	volume( μ L)	volume( μ L)
double distilled water or Milli-Q water	-	21-x	8.4-y
Template DNA	10pg-1 μ g	x	y
Primer mixture ( 10 μ M each )	0.8 μ M	4	1.6
Hi-Fi PCR Master Mix (2X)	1X	25	10
Total volume	-	50	20

**Note:** The recommended dosage of 50 μ l reaction volume for different types of templates is as follows:

Mammalian genomic DNA: 0.1-1  $\mu$ g; Escherichia coli genomic DNA: 10-100ng; Plasmid DNA: 0.1-10ng.

3. Gently blow and mix with a pipette, centrifuge at room temperature for a few seconds.
4. Put the PCR reaction liquid on the PCR instrument to start the PCR reaction.

## 二. The setting of PCR reaction parameters can be referred to as follows:

STEP1(initial denaturation): 92°C 3min

STEP2(denaturation): 92°C 30sec

STEP3(annealing): 55°C 30sec

STEP4(extension): 68°C 15-60s/kb

STEP5(cycle): Go To STEP2 for 30-35 cycles

STEP6(final extension):68°C 10min

STEP7(temporary storage): 4°C forever

**Note:** The time of the extension step needs to be set according to the length of the PCR product. For the amplification of DNA fragments smaller than 6kb, the recommended extension time is 15 seconds per kb. When amplifying DNA fragments larger than 6kb, the recommended elongation time is 1 minute per kb.

### Notes:

1. Because PCR reaction is very sensitive and can amplify the target gene sequence more than 10 million times, please pay attention to avoid contamination of trace DNA to be amplified when using Taq enzyme, and try to consider setting a blank control without template to confirm whether there is contamination of DNA to be amplified.
2. Although this product still has almost the same PCR amplification effect after 15 times of repeated freeze-thaw, it is still appropriate to avoid repeated freeze-thaw this product, repeated freeze-thaw may degrade product performance.
3. This product is only for the use of scientific researchers, can not be used for clinical diagnosis or treatment, food or medicine, shall not be stored in ordinary homes.
4. For your safety and health, please wear good clothes and disposable gloves.