

## PCR Master Mix (Green, 2X)

**Catalogue No.:** K091

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

### Storage:

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

### Introduction:

The PCR Master Mix (Green, 2X) uses a particularly convenient blue and orange dye (4kb and 50bp, respectively) and contains 2X Taq DNA Polymerase, 2X PCR Buffer, 2X dNTP and 2X Loading Buffer, only need to add appropriate primer, template and water for PCR amplification, after amplification can be directly onto the sample electrophoresis.

The product is mainly suitable for conventional PCR amplification of cDNA or genomic DNA, especially for qualitative or semi-quantitative PCR amplification, and can also be used for the amplification and cloning of DNA fragments less than 2kb in length. Fragments up to 8kb can be amplified, but it is generally better used to amplify DNA fragments below 2kb.

Electrophoretic tracer dyes of blue and orange (green overall) are added to the product, and their migration sites in the 1% agarose gel are approximately 4kb and 50bp, respectively. Electrophoretic detection can be performed directly after PCR, without the need to add sample buffer. Blue and orange tracer dyes do not affect the observation and detection of the corresponding DNA bands.

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. PCR Master Mix (Green, 2X) was melted at room temperature, gently mixed upside down and centrifuged at low speed for a few seconds.
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
PCR Master Mix (Green, 2X)	1X	25	10
Total volume	-	50	20

**Note:** The recommended dosage of 50  $\mu$ 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): 94°C 3min

STEP2(denaturation): 94°C 30sec

STEP3(annealing): 72°C 30sec

STEP4(extension): 72°C 15-60s/kb

STEP5(cycle): Go To STEP2 for 30 cycles

STEP6(final extension): 72°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, the recommended elongation time is 1 minute per kb.

## **三. Result detection**

After PCR, 5-10  $\mu$ l was directly taken for electrophoretic detection without adding sample buffer.

### **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. Taq DNA polymerase has an error rate of about  $2.2 \times 10^{-5}$  per cycle in the PCR process. For cloning DNA fragments larger than 1kb, it is recommended to use a high-fidelity DNA polymerase with a lower error rate. Taq DNA polymerase is the best choice for qualitative or quantitative PCR or RT-PCR.

3. 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.

4. 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.

5. For your safety and health, please wear good clothes and disposable gloves.