

## Tinzyme Co., Limited

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# 2×E Taq Master mix, without dye

**Product Number: PCM12** 

## **Shipping and Storage**

-20°C.

#### **Components**

| •                               |       |       |        |
|---------------------------------|-------|-------|--------|
| Component                       | PCM12 | PCM12 | PCM12  |
|                                 | (1mL) | (5mL) | (25mL) |
| 2×E Taq Master mix, without dye | 1mL   | 5×1mL | 5×5mL  |
| $\mathrm{ddH_2O}$               | 1mL   | 5×1mL | 5×5mL  |

Note: 2×E Taq Master mix, without dye contains E Taq DNA Polymerase, 3mM MgCl<sub>2</sub> and 400μM each dNTP.

### **Description**

This product is a premixed system composed of E Taq DNA Polymerase, Mg<sup>2+</sup>, dNTPs, PCR stabilizers and enhancers, with a concentration of 2×.E Taq DNA Polymerase has excellent performance of high amplification efficiency and low mismatch rate. The original MasterMix formula makes the entire reaction system very stable, with over 98% of PCR amplification being successful at once. At the same time, complex templates can also be effectively amplified, and human error and pollution can be minimized. This product does not contain dyes. After the PCR program is completed, an appropriate amount of sample loading buffer can be added as needed for electrophoresis operation. Most of the PCR products obtained by amplification have an "A" base attached to the 3 'end, so they can be directly used for T/A cloning. Mainly suitable for experiments such as routine PCR reactions and gene cloning with high fidelity requirements

## **Quality Control**

No exogenous nuclease activity was detected; no host DNA was detected by PCR; single copy genes in multiple genomes could be efficiently amplified.

#### **Protocol**

The following is an example of a PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genomic DNA as a template. The actual operation should be based on the template, the structure of the primers, and the size of the target fragment to make corresponding improvements and optimizations.

### 1. PCR reaction system

| Reagent                        | 50μL reaction   | Final Conc.          |
|--------------------------------|-----------------|----------------------|
| 2×E Taq Master mix,without dye | 25μL            | 1×                   |
| Forward primer (10µM)          | $2\mu L$        | $0.4 \mu M$          |
| Reverse primer (10µM)          | $2\mu L$        | $0.4 \mu M$          |
| Template DNA                   | <0.5μg          | $<\!\!0.5ug/50\mu L$ |
| $ddH_2O$                       | Up to $50\mu L$ |                      |

Note: For the primer concentration, please refer to the final concentration of 0.1- $1.0\mu M$  as a reference for the setting range. When the amplification efficiency is not high, the concentration of the primer can be increased; when a nonspecific reaction occurs, the concentration of the primer can be reduced, thereby optimizing the reaction system.

## 2. PCR reaction condition

| Step           | Temperature | Time  |  |
|----------------|-------------|-------|--|
| Initialization | 94°C        | 2mins |  |



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| Denaturation     | 94°C    | 30s 7           |
|------------------|---------|-----------------|
| Annealing        | 55-65°C | 30s 25-35cycles |
| Elongation       | 72°C    | $_{30s}$ $\Box$ |
| Final elongation | 72°C    | 2mins           |

Note: 1) In general, the annealing temperature is 5°C lower than the melting temperature (Tm) of the primer. When the desired amplification efficiency cannot be obtained, the annealing temperature is appropriately lowered; when non-specific reactions occur, the annealing temperature is increased, thereby optimizing the reaction conditions.

- 2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of E Taq DNA Polymerase is 2kb/min.
- 3) The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too small, the amount of amplification is insufficient; if the number of cycles is too big, the probability of mismatch increases, and the non-specific background is severe.