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2×E-Taq PCR Master Mix Instructions

Cat No.: SLPCR241

Product Composition

Specification	5ml	25ml	40ml
2×E-Taq PCR Master Mix	1 ml×5	5 ml×5	1 ml×40
ddH_2O	1 ml×5	5 ml×5	1 ml×40
Instruction Manual	1 copy	1 copy	1 copy

Note: 2×E-Taq PCR Master Mix contains 3 mM Mg^{2+}.

Product Storage and Shelf Life

- Store at -20°C, and the shelf life is more than two years.
- Store at 2 - 8°C, and the shelf life is 6 months. Repeated freezing and thawing up to 16 times will not affect the performance.

Product Introduction

2×E-Taq PCR Master Mix is an optimized 2X concentrated PCR premix. It is suitable for PCR with high requirements for fidelity and long amplified fragments. It can amplify fragments up to 10 kb from complex genomic DNA. The PCR enhancer and protein stabilizer work together to improve PCR efficiency and sensitivity, making it very suitable for the amplification of low-copy templates. The product is easy to use. Just take 0.5 times the volume of the PCR system of 2×E-Taq PCR Master Mix, add primers and templates, and make up the volume with ddH_2O.

2×E-Taq PCR Master Mix contains E-Taq DNA Polymerase. Most of the amplified target products have an A base attached to the 3' end, which can be directly cloned into the T-Vector. The product contains a blue electrophoresis indicator dye, and the amplified products can be directly detected by agarose gel electrophoresis.

Components of the PCR System

1. **Purity of Template DNA**: Many residual nucleic acid extraction reagents can affect the PCR reaction, including proteases, protein denaturants (such as SDS, guanidine salts), high-concentration salts (KAc, NaAc, sodium caprylate, etc.), and high-concentration EDTA. For templates with low purity (such as those obtained by boiling), the usage amount should not exceed 1/10 of the PCR reaction system (for example, in a 50 μl reaction system, the volume of the added template should not exceed 5 μl). If the purity of the template DNA is too poor, the Sunlong Biotech DNA purification kit (Cat. No.EXD0215) can be used to purify and concentrate the template DNA. The usage amount of the template purified by the Sunlong Biotech DNA purification kit can be up to 1/2 of the volume of the PCR reaction system.

2. **Usage Amount of Template DNA**: Trace amounts of DNA can also be used as PCR templates. However, to ensure the stability of the reaction, it is recommended to use more than 10^4 copies of the target sequence as the template in a 50 μl system. The recommended usage amounts of template DNA are as follows:
    - Human Genomic DNA: 0.05 μg - 0.5 μg/50 μl PCR reaction system
    - Escherichia coli Genomic DNA: 10 ng - 100 ng/50 μl PCR reaction system
    - λ DNA: 0.5 ng - 5 ng/50 μl PCR reaction system
    - Plasmid DNA: 0.1 ng - 10 ng/50 μl PCR reaction system

    If the amplified product is used as a template for re-amplification, it should be diluted at least 1,000 - 10,000 times before use. Otherwise, smeared bands or non-specific bands may appear.
3. **Primer Concentration**: Generally, the concentration of each primer is prepared at 10 μM (50×), and the working concentration is 0.2 μM. Excessive primers may cause non-specific amplification, while too few primers may reduce the amplification efficiency.

PCR Parameter Settings

1. **Pre-denaturation**: Generally, pre-denaturation is carried out at 94°C for 1 - 5 minutes. Excessively high denaturation temperature or an overly long denaturation time will reduce the activity of E-Taq enzyme.
2. **Annealing**: The annealing temperature is the key to PCR. Too high annealing temperature may reduce the yield, while too low annealing temperature may cause primer dimers or non-specific amplification. For the first attempt at PCR amplification, it is recommended to try an annealing Tm 5°C lower than the Tm (if the Tm values of the two primers are different, refer to the lower Tm). Generally, primer synthesis companies will provide the Tm of the synthesized primers. The Tm of the primers can also be estimated according to the formula: Tm = 2°C× (A + T)+4°C × (G + C). The optimal annealing temperature needs to be determined by gradient PCR.
3. **Extension**: The extension temperature is usually 72°C. The extension time depends on the length of the target DNA fragment, calculated as 1 kb/min. An excessively long extension time may lead to an increase in non-specific products. After the cycling is completed, continue the extension for 5 - 10 minutes to obtain complete double-stranded products.
4. **Number of Cycles**: Generally, 25 - 35 cycles are used. For low-copy templates, the number of cycles can be appropriately increased.An excessively high number of cycles may increase non-specific amplification and reduce specific products.

Operation Steps

1. Thaw 2×E-Taq PCR Master Mix, ddH_2O, template DNA, and primers at room temperature and place them on ice.

2. After thawing, mix each component evenly by inverting it up and down. Prepare the PCR reaction system according to the following composition:

◦ 2×E-Taq PCR Master Mix:	25 μl
◦ Primer 1 (10 μM):	1 μl
◦ Primer 2 (10 μM):	1 μl
◦ Template:	n μl
◦ ddH_2O:	(23 - n) μl
◦ Total:	50 μl

Note: The above example shows the components added to a 50 μl reaction system. If a reaction system of other volumes is required, increase or decrease each component proportionally.

3. Gently flick the PCR reaction tube to mix well, and centrifuge at low speed for a few seconds to make the solution settle to the bottom of the tube.

4. Example of PCR reaction cycle settings:

◦ 94°C for 3 minutes
◦ 94°C for 30 seconds
◦ ※ 55°C for 30 seconds } (30 Cycles)
◦ § 72°C for 1 minute
◦ 72°C for 5 minutes

※ Use the actual optimal annealing temperature. § Calculate at 1 kb/min.

5. **Result Detection**: Take 5 - 10 μl of the amplified product and directly perform agarose gel electrophoresis for detection.

Relationship between agarose gel concentration and the optimal resolution range of linear DNA:

Agarose Concentration	Optimal Resolution Range of Linear DNA
0.5%	1,000 - 30,000
0.7%	800 - 12,000
1.0%	500 - 10,000
1.2%	400 - 7,000
1.5%	200 - 3,000
2.0%	50 - 2,000

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