SIMGEN

Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

2×E-Taq PCR Master Mix Instruction

Composition

Cat. No.	7007001	7007005	7007025	7007040
2×E-Taq PCR Master Mix	1 ml	1 ml×5	5 ml×5	1 ml×40
ddH_2O	1 ml	1 ml×5	5 ml×5	1 ml×40
Instructions	1	1	1	1

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

Storage

It can be stored at -20℃ for more than 2 years. It can be stored at 2~8℃ for 6 months. Repeated freezing and thawing 16 times will not affect the use effect.

Technical support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

2×E-Taq PCR Master Mix is an optimized double concentration PCR premix. It is suitable for PCR with high fidelity requirements and long amplification fragments, which can amplify fragments up to 10 kb from complex genomic DNA. PCR enhancers and protein stabilizers work together to improve PCR efficiency and sensitivity, making them ideal for low-copy template amplification. The product is easy to use. It only needs to take a 2×E-Taq PCR Master Mix of 0.5 times the volume of the PCR system, add primers and templates, and make up the volume with ddH_2O.

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

Components of the PCR system

1. Purity of template DNA: Many residual nucleic acid extraction reagents can affect the PCR, including proteases, protein denaturants (e.g., SDS, guanidine salts), high concentrations of salts (KAc, NaAc, sodium caprylate, etc.), and high concentrations of EDTA. Do not use more than 1/10 of the PCR reaction for less pure templates (e.g., 5 µl of the sample for 50 µl reaction system). If the template DNA purity is too poor, the Simgen DNA Purification Kit (Cat. No.2101050) is recommended to purify and concentrate the template DNA, and the amount of template used after purification can be added as much as 1/2 of the volume of the PCR system.
2. Template DNA dosage: A very small amount of DNA can also be used as a PCR template, but to ensure the stability of the reaction, it is recommended to use more than 10^4 copies of the target sequence as a template for the 50 µl system. Recommended dosage of template DNA:

Human Genomic DNA:	0.05 µg~0.5 µg/50 µl PCR system
E. coli genomic DNA:	10 ng~100 ng/50 µl PCR system
λ DNA：	0.5 ng~5 ng/50 µl PCR system
Plasmid DNA：	0.1 ng ~ 10 ng/50 µl PCR system

   If the amplification product is to be used as a template for reamplification, the amplification product should be diluted at least 1,000 to 10,000-fold before using it as a template, otherwise smeared bands or non-specific bands may occur.
3. Primer concentration: Typically, each primer is prepared at a concentration of 10 µM (50×) and a working concentration of 0.2 µM. Too much primer may result in non-specific amplification, and too little primer may reduce amplification efficiency.

Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

PCR Parameter Settings

1. Initial denaturation: the general Initial denaturation is 94°C, 1~5 min. Denaturation temperature is too high or too long time will lose the activity of E-Taq enzyme.
2. Annealing: Annealing temperature is the key in PCR, too high a temperature may reduce yield, and too low a temperature may produce primer-dimers or non-specific amplification. For the first attempt at PCR amplification, it is recommended to try a lower than 5 °C Tm (if the two primer Tm are different, refer to the lower Tm) as the annealing temperature. Generally, primer synthesis companies will provide the Tm of the synthesized primers, and the primer Tm can also be estimated according to this 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 typically 72°C, and the length of Extension time depends on the length of the DNA fragment of interest, and the required Extension time is calculated at 1 kb/min, which may lead to a non-specific increase. After the end of the cycle, continue to extend for 5~10 min to obtain the complete double-stranded product.
4. Number of cycles: Generally, 25~35 cycles are used, and the number of cycles can be increased appropriately with a low copy template. Excessive number of cycles may increase non-specific amplification and decrease specific products.

Protocol

1. Thaw 2×E-Taq PCR Master Mix, ddH_2O, template DNA, and primer at room temperature and place on ice.
2. Invert the thawed components upside down and mixed evenly, and the PCR system is prepared according to the following table:

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 examples are the components added to the 50 µl reaction system. If other volumes of the reaction system are required, please increase and decrease the components in proportion.

3. Flick the PCR tube to mix thoroughly and spin down the solution to the bottom of the tube.
4. Example of PCR reaction cycle step

Step	Temperature(℃)	Time	Number Of Cycles
Initial Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	30
Annealing	※55°C	30 sec	
Extension	§ 72°C	1 min	
Final Extension	72°C	5 min	1

※ The actual optimum annealing temperature shall prevail.

§ Calculated in 1 kb/min.

5. Results: 5-10 µl of the amplified product was directly detected by agarose electrophoresis.

The relationship between the concentration of agarose gel and the best resolution range of linear DNA:

Agarose concentration	Optimal linear-shaped DNA resolution range
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