

2×Taq Plus PCR Master Mix instructions

Composition

Cat. No.	7005100	7005500
2×Taq Plus PCR Master Mix	1 ml	1 ml×5
ddH ₂ O	1 ml	1 ml×5
Instructions	1	1

Storage

The storage period is more than 2 years at -20°C, and the validity period is 6 months when stored at 2~8°C. Repeated freeze-thaw 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

The 2×Taq Plus PCR Master Mix is an optimized two-fold concentration PCR master mix. Ideal for high-fidelity, long-amplified PCR that can amplify fragments up to 10 kb from complex genomic DNA. Taq enzyme antibodies, PCR enhancers, and protein stabilizers work synergistically to improve PCR efficiency and sensitivity, making them ideal for low-copy template amplification. The product is easy to use, just take 2×Taq Plus PCR Master Mix 0.5 times of the volume of the PCR system, add primers and template, and make up the volume with ddH₂O.

2×Taq Plus PCR Master Mix contains Taq enzyme and a certain proportion of pfu DNA Polymerase, and most of the amplified target products have an A base attached to the 3' end, which can be cloned directly into T-Vector. The product contains two types of indicator dyes for electrophoresis, red and yellow. They do not inhibit PCR and do not affect EB color development, and their relative electrophoresis migration distances are shown in the following table.

Gel Concentration	Red Dye	Yellow Dye
0.8%	2000 bp	~80 bp
1.0%	1500 bp	~40 bp
1.5%	1000 bp	~20 bp
2.0%	500 bp	<10 bp
2.5%	350 bp	<10 bp
3.0%	200 bp	<10 bp

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 (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 PCR 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 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 10⁴ 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.

PCR parameter settings

1. Initial denaturation: generally, initial denaturation is 94°C, 1~5 min. Denaturation temperatures that are too high or too long can result in loss of Taq enzyme activity.
2. Annealing: Annealing temperature is 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 T_m (if the two primer T_m are different, refer to the lower T_m) as the annealing temperature. Generally, primer synthesis companies will provide the T_m of the synthesized primers, and the primer T_m can also be estimated according to this formula: T_m = 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×Taq Plus PCR Master Mix, ddH₂O, template DNA, and primers at room temperature and place on ice.
2. After thawing, the components were inverted up and down to mix evenly, and the PCR system was prepared according to the following table.

2×Taq Plus PCR Master Mix	25 μl
Primer 1 (10 μM)	1 μl
Primer 2 (10 μM)	1 μl
template	n μl
ddH ₂ O	(23-n) μl
Total	50 μl

* Note: The above example is for the addition of components to a 50 μl reaction system, if additional volumes of the reaction are required, please increase or decrease the components proportionally.

3. Flick the PCR tube to mix well, and spin the solution to the bottom of the tube.
4. Example of PCR reaction cycle setup

Step	Temperature(°C)	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 optimal annealing temperature shall prevail.

§ Calculated at 1kb /min.

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

Relationship between agarose gel concentration and the optimal 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