SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

2 ×Taq PCR Master Mix

Product Formation

Cat .No .	SLPCR229-1	SLPCR229-2
2×Taq PCR Master Mix	1 ml	1 ml ×5
ddH2O	1 ml	1 ml ×5
instructions	A	A

Product storage and shelf-life

The shelf age below 20℃ is more than two years; 2~8℃ for 6 months. Repeated freezing and thawing does not affect the use.

Product presentation

2 Taq PCR Master Mix is an optimized twice-concentration premixture of PCR. Suitable for conventional PCR, either to amplify fragments up to 4 kb from complex genomic DNA or up to 5 kb from λ DNA. PCR enhancers and protein stabilizers synergistically improve PCR efficiency and sensitivity and are ideal for low-copy template amplification. The product is easy to use, taking only 2 Taq PCR Master Mix of the volume of the PCR system, adding primers and templates to ddH2O can make up the volume.

The target product amplified by this kit has an A base at the 3 ′ end, which can be directly cloned in T-Vector. The product contains two electrophoretic indicator dyes, which do not inhibit PCR nor affect EB color development. The relative migration distance of electrophoresis is shown in Table:

Relative migration distance between agarose gel concentration and dye

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

The pcR system components

1. **Purity of template DNA:** Many residual nucleic acid extraction reagents can affect the PCR reaction, including proteases, protein denatants (such as SDS, guanidine salt), high concentration salt (KAc, NaAc, sodium, etc.) and high concentration EDTA. The amount of the template with low purity (such as that obtained by boiling) should not exceed 1 / 10 of the PCR reaction system (for example, the volume of the template in the 50 μ l reaction system should not exceed 5 μ l). If the template DNA is poorly pure, use the Sunlong Biotech DNA purification kit (Cat No.:PU002) The template DNA was purified and concentrated, and the purified template could be used for as much as 1 / 2 of the volume of the PCR reaction system.

2. **Amount of template DNA:** very trace DNA can also be used as a PCR touch plate, but in order to ensure the stability of the reaction, 50 μ l system is recommended 104 Copies above the target sequence were used as a template. Recommended use of the template DNA:

Human genomic DNA:	0.05 μg ~0.5 μg / 50 μl PCR reaction system
Genomic DNA of E. coli:	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 template, the amplified product should be diluted at least 1,000 to 10,000 times before use as template, otherwise smear bands or no specific bands may occur.

3. **Primer concentration:** generally the concentration of each primer is 10 μ M (50),and the working concentration is 0.2 μ M. Nonspecific amplification may occur in excess of primers, and too few primers may reduce the amplification efficiency.

PCR parameter setting

1. **Predegeneration:** the general predegeneration is 94℃, 1~5 min. Too high or too long can lose the activity of the Taq enzyme.
2. **Annealing:** The annealing temperature is the key of PCR. Too high temperature may reduce the yield, and too low temperature may produce primer dimer or non-specific amplification. The initial attempt of PCR amplification recommends direct 55℃ or 5℃ below Tm (if different Tm, refer to lower Tm) as the annealing temperature. The general primer synthesis company will provide the Tm of the synthesized primer, or the primer Tm can be estimated according to this formula: Tm = 2℃ (A + T) + 4℃ (G + C). The optimal annealing temperature requires a gradient PCR determination.
3. **Extension:** The extension temperature is usually 72℃, and the length of extension depends on the length of the target DNA fragment. The extension time is calculated at 1 kb /min. A long time may lead to a non-specific increase. After the cycle, the was continued for 5 to 10 min to obtain a complete duplex product.
4. **Number of cycles:** 25~35 cycles are generally used, and the low-copy template can increase the number of cycles appropriately. Excessive cycle number may increase nonspecific amplification and decrease specific products.

Operating steps

1. will 2 Taq PCR Master Mix, ddH2O, template DNA, and primers were thawed at room temperature and placed on ice.

2. Turn the thawed components over and mix them evenly,Compounding according to the following composition PCR reaction system:

2×Taq PCR Master Mix	25 μ l
Primer 1 (10 μ M)	1 μl
Primer 2 (10 μ M)	1 μl
Template	n μ l
ddH2O	(23-n) μ l
Total	50 μ l*

* The above example is the fraction added to the 50 μ l reaction system. If another volume of reaction system is required, please add or subtract each fraction proportional.

3. Mix the PCR reaction tube well, centrifuge at low speed for several seconds to make the solution settle to the bottom of the tube.

4. Example of the PCR reaction cycle setting

94℃ 3 min

94℃ 30 sec

※55℃ 30 sec 35 Cycles } 35 Cycles

§ 72℃ 1 min

72℃ 5 min

※The actual optimal annealing temperature shall prevail.
§ Calculated at 1 kb / min.

For amplified target fragments below 300bp, a two-step method can be used to save amplification time:

94℃ 3 min

94℃ 20 sec } 35 Cycles

60℃ 1 min

72℃ 5 min

5. Results detection: 510 μ l of the amplification product was tested directly by agarose electrophoresis.Relationship between agarose gel concentration and the best resolution range of linear DNA:

The concentration of agarose	Optimum linear 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