Drop PCR Mix (Dye)

Catalog Number:

CW2599M (5 ml)
CW2599L (25 ml)

Storage Condition:

-20°C; For frequent uses, store at 2-8°C.

Kit Components:

Component	CW2599M (5 ml)	CW2599L (25 ml)
Drop PCR Mix (Dye)	5 ml	5× 5 ml

Product Introduction:

The Drop PCR Mix (Dye) includes all the elements required for PCR reaction, PCR stabilizers and enhancers. It has a high degree of tolerance for the concentration of each component in the PCR reaction. Without pipettes, the product can be added dropwise directly to the PCR tube with template and primers already added, to complete the reaction preparation.

This product is very stable and can be stored at 2-8°C for 6 months after first use. The DNA polymerase in this product is a hot-start polymerase, which can effectively reduce the amplification of non-specific PCR products. The preparation of the PCR reaction can be performed at room temperature. The first step of the PCR program must be at 95°C for 10 minutes. This product has been added with blue dye, and the PCR product can be directly loaded on electrophoresis gel after the reaction. Most of the PCR products have an "A" base attached to the 3' end, and therefore can be directly used for T/A cloning.

Quality Control:

No exogenous nuclease activity was detected; no host residual DNA was detected by the PCR; a single copy gene can be efficiently amplified; the activity was not significantly changed after storage at 2-8°C for 6 months.

Protocol:

This product is packaged as a drop bottle. When using it, remove the cap and invert the bottle. Face the bottle toward the PCR tube with template and primers already added. Squeeze the bottle gently with the index finger and thumb to add a drop to the PCR reaction.

In the normal push, the average droplet volume is about 25 µl. The droplet volume may be different due to the difference of pushing strength. But it works well for most PCR reactions.

1. PCR reaction system:

Reagent	Volume
Drop PCR Mix (Dye)	1 drop
Forward Primer, 10 µM	1 µl
Reverse Primer, 10 µM	1 µl
DNA template	≤ 4 µl

Note:

1) The concentration of primers should be 10 µM. If the amplification efficiency is low, the concentrations of the primers can be increased; If a non-specific reaction occurs, the concentration of the primers can be decreased, thus optimizing the reaction system.

2) The amount of PCR template should be adjusted. In general, when the template is plasmid DNA, it should be between 1 pg to 10 ng; when the template is bacterial genomic DNA, 1 ng to 100 ng is suitable; when the template is eukaryotic genomic DNA, 10 ng to 300 ng is preferable. Excessive template can inhibit the PCR reaction, and it is easy to increase the level of other inhibitors to affect the amplification.

2. PCR reaction program:

Step	Temperature	Time	Cycles
Initialization	95°C	10 min	1
Denaturation	95°C	30 sec	30-40
Annealing	55-65°C	30 sec	
Extension	72°C	1 min	
Final Extension	72°C	5 min	1
Hold	4-12°C		

Note:

1) The product contains a hot-start DNA polymerase which must be activated at 95°C for 10 minutes.

2) 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 can be appropriately lowered; when non-specific reactions occur, the annealing temperature can be increased, thereby optimizing the reaction conditions.

3) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of this product is 1 kb/min.

4) 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. Therefore, the number of cycles should be reduced as much as possible yet ensuring the yield of the product.