5X DNA Annealing Buffer

Product description

DNA Annealing Buffer is designed for conventional DNA oligo annealing. It effectively prevents hairpin structure formation of single DNA oligos, and the annealed double-stranded oligos can be directly used for ligation with restriction enzyme-digested and purified plasmids. The protocol is simple: mix the DNA oligos with 5X DNA Annealing Buffer at a proper ratio, place in a PCR thermal cycler, and the annealing reaction will be completed in approximately 90 minutes.

Composition and storage conditions

Components \ Size	1 mL	Storage
5×DNA Annealing Buffer	1 mL	-20°C

Shipping: Blue Ice **Shelf life:** 12 months

Experimental operation

1. Prepare the DNA oligos to be annealed at 50 μM using sterile nuclease-free water or ddH_2O. Thaw and mix 5X DNA Annealing Buffer completely before use.

2. Set up the annealing reaction as follows:

Reagent	Volume (μL)
5×DNA Annealing Buffer	20
DNA oligo A (50 μM)	20
DNA oligo B (50 μM)	20
Nuclease-Free Water	40
Total Volume	100

***Note:** If the PCR thermal cycler is not equipped with a heated lid, add a drop of mineral oil to prevent evaporation.

3. Program the PCR thermal cycler as below:

Step	Temperature	Time	Note
1	95℃	2 min	Oligo denaturation
2	Decrease 0.1℃ every 8 sec until 25℃	~ 90 min	Annealing
3	4℃	Hold	Storage

***Note:** Temperature decreases by 0.1℃ every 8 sec until reaching 25 ℃. Taking the Bio-Rad T100 as an example, the program is set as follows: 1. 95℃, 2:00; 2. 95℃, 0.08, -0.1℃ per cycle; 3. GOTO step 2, 700X; 4. 4℃, ∞. If the PCR instrument does not support a temperature drop of 0.1 ℃ per cycle, an alternative protocol can be set with 1℃ decrease every 90 sec: 1. 95℃, 2:00; 2. 95℃, 1:30, -1℃ per cycle; 3. GOTO step 2, 70X; 4. 4℃, ∞

4. The annealed product can be used directly for downstream reactions or stored at -20°C for later use.

***Note:** If the annealed product is to be used for restriction digestion or other enzymatic reactions, purification with a purification kit is recommended. Alternatively, ensure the annealed product constitutes no more than 5% of the total reaction volume to avoid interference from the annealing buffer.

Notes

1. This product is only for annealing of DNA oligos; it is not suitable for RNA oligos.
2. This product is for research use only.

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