

## 5X RNA Annealing Buffer

### Product description

RNA Annealing Buffer is designed for routine RNA oligo annealing, which effectively prevents RNA oligos from forming self-complementary hairpin structures. This kit features a simple workflow. Simply mix the RNA oligos to be annealed with RNA Annealing Buffer at the recommended ratio, place the sample in a PCR instrument, and the annealing procedure can be completed in approximately 60 minutes.

### Composition and storage conditions

Components	Size	1 mL	Storage
5×RNA Annealing Buffer		1 mL	-20°C
Shipping: Blue Ice		Shelf life: 12 months	

### Experimental operation

1. Prepare the RNA oligos to be annealed at 200  $\mu\text{M}$  using sterile nuclease-free water or ddH<sub>2</sub>O. Thaw and mix 5X RNA Annealing Buffer completely before use.
2. Set up the annealing reaction as follows:

Reagent	Volume ( $\mu\text{L}$ )
5×RNA Annealing Buffer	20
RNA oligo A (200 $\mu\text{M}$ )	25
RNA oligo B (200 $\mu\text{M}$ )	25
Nuclease-Free Water	30
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:

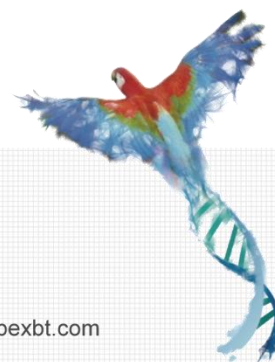
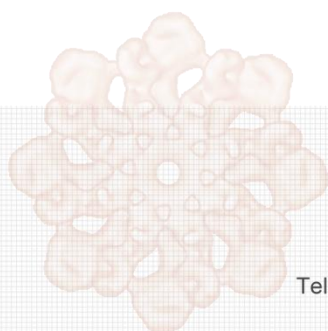
Step	Temperature	Time	Note
1	95°C	1 min	Oligo denaturation
2	Decrease 0.1°C every 5 sec until 25°C	~ 60 min	Annealing
3	4°C	Hold	Storage

**\*Note:** Temperature decreases by 0.1°C every 5 sec until reaching 25°C. Taking the Bio-Rad T100 as an example, the program is set as follows: 1. 90°C, 2:00; 2. 90°C, 0.05, -0.1°C per cycle; 3. GOTO step 2, 650X; 4. 4°C, ∞. If the PCR instrument does not support a temperature drop of 0.1 °C per cycle, an alternative protocol can be set with 1°C decrease every 60 sec: 1. 90°C, 1:00; 2. 90°C, 1:00, -1°C per cycle; 3. GOTO step 2, 65X; 4. 4°C, ∞

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

## Notes

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



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