# 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 μM using sterile nuclease-free water or dd$H_2O$. Thaw and mix 5X RNA Annealing Buffer completely before use.

2. Set up the annealing reaction as follows:

| Reagent | Volume (μL) |
|---|---|
| 5×RNA Annealing Buffer | 20 |
| RNA oligo A (200 μM) | 25 |
| RNA oligo B (200 μM) | 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℃ | 1 min | Oligo denaturation |
| 2 | Decrease 0.1℃ every 5 sec until 25℃ | ~ 60 min | Annealing |
| 3 | 4℃ | Hold | Storage |

***Note:** Temperature decreases by 0.1℃ every 5 sec until reaching 25℃. Taking the Bio-Rad T100 as an example, the program is set as follows: 1. 90℃, 2:00; 2. 90℃, 0.05, -0.1℃ per cycle; 3. GOTO step 2, 650X; 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 60 sec: 1. 90℃, 1:00; 2. 90℃, 1:00, -1℃ per cycle; 3. GOTO step 2, 65X; 4. 4℃, ∞

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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