

## RNA Extraction Buffer (Chloroform Substitute)

### R749970

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**Storage** Store at room temperature in the dark.

**Shipping** Shipped under normal conditions. Please store under the specified storage conditions immediately upon receipt.

#### Introduction:

Chloroform substitutes are mainly used to replace chloroform reagents in RNA extraction processes related to Trizol and other RNA extraction reagents. This product has low toxicity and can be used as a substitute for chloroform. The usage method is the same as chloroform; It will not have any adverse effects on the quality or quantity of isolated RNA.

#### Instructions for Use :

1. After sample pretreatment, add 1 mL Trizol reagent to lyse the sample, and incubate at room temperature for 5 minutes.
2. Add 0.1 mL chloroform substitute, shake thoroughly to mix, then incubate for 3 minutes.
3. Centrifuge the sample at  $12,000 \times g$  for 15 minutes at  $4\text{ }^{\circ}\text{C}$ .
4. Transfer approximately 500  $\mu\text{L}$  of the upper aqueous phase to a new sample tube. After centrifugation, the mixture separates into three layers: the lower organic layer, the intermediate layer and the colorless upper aqueous phase. RNA is present in the upper aqueous phase.
5. Add isopropanol of the same volume as the collected aqueous phase, invert to mix well, and incubate at room temperature for 10 minutes.
6. Centrifuge at  $12,000 \times g$  for 10 minutes at  $4\text{ }^{\circ}\text{C}$ , then discard the supernatant.
7. Add 1 mL of 75% ethanol to wash the pellet. Centrifuge at  $12,000 \times g$  for 3–5 minutes at  $4\text{ }^{\circ}\text{C}$  and discard the supernatant. Repeat this step once.

Note: Briefly centrifuge to collect residual liquid, then remove it with a pipette tip. Take care not to aspirate the pellet.

8. Air-dry the pellet at room temperature for 3–5 minutes, then add 20–50  $\mu\text{L}$  RNase-free water to fully dissolve the pellet. (Dissolution can be accelerated by water bath incubation at  $55\text{ }^{\circ}\text{C}$ .)

Note: Do not over-dry the pellet, otherwise it will be difficult to dissolve.

#### Precautions :

1. If the usage volume per time is small, please aliquot the reagent appropriately before use to avoid contamination.
2. For your safety and health, please wear a lab coat and disposable gloves during operation.