

# RNA purification

## Materials:

- *EasyPure*® RNA Purification Kit(Transgene, catalog no. ER701-01)
- Sterile and nuclease-free 1.5 or 2.0-mL Eppendorf tubes, PCR tubes or multi-well plates
- Nuclease-free, molecular biology-grade water
- 100% ethanol
- $\beta$ -mercaptoethanol
- chloroform

## Procedure:

Please add 32ml of 100% ethanol to WB12 before use. All centrifugation is performed at room temperature.

Reagents provided by customers: chloroform, 96%-100% ethanol

1. Transfer:  $\leq 100\mu\text{g}$  RNA sample into a microcentrifuge tube and supplement to 100ul with RNase-free water. Add 350ul of BB12 (add 10ul  $\beta$ -mercaptoethanol for per ml BB12, and it must be freshly prepared before use). Mix thoroughly by inverting or vortexing.
2. Add 900ul of 96%-100% ethanol (precipitates may form at the stage). Mix thoroughly by inverting or vortexing.
3. Transfer half volume of solution and precipitates together to a spin column. Centrifuge at 12,000 $\times$ g for 30 seconds at room temperature. Discard the flow through.
4. Repeat step 3 with the remaining half volume of the solution.
5. Add 500ul of WB12 (Check to be sure ethanol has been added) into the spin column. Centrifuge at 12,000 $\times$ g for 30 seconds at room temperature. Discard the flow through.
6. Repeat step 5 once.
7. Centrifuge at 12,000 $\times$ g for 2 minutes at room temperature. Air-dry the column matrix for several minutes.
8. Place the spin column into a clean 1.5ml RNase-free tube. Add 15-50ul RNase-free Water into the spin column matrix and incubate at room temperature for 1 minute.
9. Centrifuge at 12,000 $\times$ g for 1 minute to elute RNA.
10. Store the purified RNA at -80C.