

For research use only

Version Number: 2.0

## Animal Total RNA Isolation Kit

For total RNA purification from animal tissues and cells (Animal tissues  $\leq 20$  mg; Cells  $\leq 5 \times 10^6$ )

| Kit composition               | RE-03011 | RE-03014 |
|-------------------------------|----------|----------|
|                               | 50 T     | 200 T    |
| Buffer RL1*                   | 25 mL    | 100 mL   |
| Buffer RL2                    | 15 mL    | 60 mL    |
| Buffer RW1                    | 25 mL    | 100 mL   |
| Buffer RW2                    | 24 mL    | 96 mL    |
| RNase-Free ddH <sub>2</sub> O | 10 mL    | 40 mL    |
| RNA-Only Column               | 50       | 200      |
| DNA-Cleaning Column           | 50       | 200      |
| Instruction                   | 1        | 1        |

\*Please wear gloves and take protective measures during the operation as Buffer RL1 and Buffer RW1 contain irritating chaotropic salts.

### Product introduction

This kit adopts centrifugal columns and formulas developed by Foregene, and can extract high purity and quality of total RNA from various animal tissues with high efficiency. DNA-Cleaning column allows for easy separation of supernatant from lytic cell content and removes genomic DNA. RNA-only Column can efficiently bind to RNA with the help of the uniquely formulated buffer. It is simple and time-saving, while processing a large number of samples.

The entire system is RNase-free. Buffer RW1 and Buffer RW2 make the obtained RNA free from contamination by proteins, DNA, ions, and organic compounds.

### Product information

|                |                     |                        |   |
|----------------|---------------------|------------------------|---|
| Format         | Spin column         | Purification component | Foregene column, reagent                  |
| Flux           | 1-24 samples        | Time per prep          | ~30 min (24 samples)                      |
| Centrifuge     | Desk centrifuge     | Pyrolysis separation   | Centrifugal separation                    |
| Sample         | Animal tissue; cell | Samples amount         | Tissue:10-20 mg; Cell:(1-5) $\times 10^6$ |
| Elution volume | 50-200 $\mu$ L      | Maximum loading volume | 850 $\mu$ L                               |

### Storage

This kit can be stored for 24 months at room temperature (15-25°C) and in dry environment; For further storage, please place it in 2-8°C environment. Buffer RL1 can be stored for a month at 4°C after adding 2-hydroxy-1-ethanethiol (optional).

Note: If stored at a low temperature, it is easy to form the precipitate. Please place the kit solution at room temperature (15-25°C) for a few minutes before use, or preheat it in a 37°C water bath for 10 minutes to dissolve the precipitate before use.

### RNA yield and purity

The yield of RNA purified by Animal Total RNA Isolation Kit is related to initial tissue amount, freshness, preservation time and operation. The following is the yield and purity of RNA by using the kit to extract RNA from various animal tissue. In practice, there might be small differences.

| Tissue Type              | RNA yield ( $\mu$ g) | OD260/280 | OD260/230 |
|--------------------------|----------------------|-----------|-----------|
| Heart (10 mg )           | 5-20                 | 1.8-2.1   | 1.8-2.1   |
| Liver (10 mg )           | 40-60                | 1.8-2.1   | 1.8-2.1   |
| Spleen (10 mg )          | 30-50                | 1.8-2.1   | 1.8-2.1   |
| Kidney (10 mg )          | 25-35                | 1.8-2.1   | 1.7-2.0   |
| Brain (10 mg )           | 3-10                 | 1.8-2.1   | 1.5-1.7   |
| Cultured cell ( $10^6$ ) | 10-30                | 1.8-2.1   | 1.8-2.1   |

### Note: (Be sure to read the notes carefully before using)

- ◆ All procedures are carried out at room temperature(15-25°C), including centrifugation. **Do not use ice bath or centrifuge at low temperature (4°C).**
- ◆ The sample should avoid repeated freezing and thawing, otherwise it will lead to the degradation of the extracted RNA and the yield of RNA will also decrease.
- ◆ The yield and quality of RNA is tightly related to sample size and volume of elution. For every 500  $\mu$ L Buffer RL1, the recommended maximum tissue amount is 10~20mg.
- ◆ Before using the kit, please add 2-hydroxy-1-ethanethiol to Buffer RL1 (10  $\mu$ L 2-hydroxy-1-ethanethiol per 1 mL Buffer RL1). Buffer RL1 can be stored at 4°C for 1 month after adding 2-hydroxy-1-ethanethiol. If the extracted RNA is not used to clone full-length cDNA, but only used for other downstream operations such as qPCR or sequencing analysis, it is not necessary to add 2-hydroxy-1-ethanethiol, and the result will not be affected.

- ◆ Before using the kit, please add anhydrous ethanol to buffer RL2 and Buffer RW2 referring to the label on the reagent bottle for the dosage.
- ◆ The volume of elution should be no less than 50  $\mu\text{L}$ , otherwise it will influence RNA yield.
- ◆ Please check if there is any precipitate in Buffer RL1 and Buffer RW1. If the precipitate can be seen after storage at low temperature, the solution should be placed at room temperature (15-25°C) or 37°C for a period of time. Use the solution after dissolving and blending.

## Procedure (Do Not ice bath or low temperature (4°C) centrifugation)

Before using, add ethanol to Buffer RL2 and Buffer RW2 referring to the label on the bottle.

1. Please lyse cells based on different types and sources.

- a) Animal tissue:

Homogenization: Add 10-20 mg tissue into 500  $\mu\text{L}$  Buffer RL1 and grind it evenly with glass or electric homogenizer.

Note: The amount of tissue should not exceed 20 mg, or it may cause the blockage of DNA-Cleaning Column, resulting in the decline of RNA quality.

- b) Cultured cells:

- 1) Adherent cells: Use Buffer RL1 directly to digest and lyse cells. Or add Buffer RL1 to the collected cells by centrifugation (500  $\mu\text{L}$  Buffer RL1 for every  $1-5 \times 10^6$  cells), and then repeatedly pipette the cells (Until aggregated cells cannot be seen).

- 2) Suspension cells: Collect cells by centrifugation, then add Buffer RL1 (500  $\mu\text{L}$  Buffer RL1 for every  $1-5 \times 10^6$  cells), pipette the cells repeatedly (Until aggregated cells cannot be seen).

Note: The RNA in Buffer RL1 can resist RNase degradation. The cells or tissues after being lysed by Buffer RL1 can be stored at room temperature for approximately 24 hours if they are not used immediately. Also, they can be stored at 4°C for about 1 week, and for longtime storage, please keep them in -80°C. Heat them in 37°C or leave them at room temperature before use.

2. Transfer the lysate to DNA-Cleaning Column (put DNA-Cleaning Column into collection tube), and centrifuge at 12,000 rpm (~13,400  $\times g$ ) for 2 minutes. Remove DNA-Cleaning Column and keep the supernatant in the collection tube.

Note: If the amount of tissue is more than 20 mg or there are many lumps after grinding, transfer the lysate to a 1.5 mL centrifugal tube, centrifuge at 12,000 rpm (~13,400  $\times g$ ) for 5 minutes, then proceed to step 2. If there is precipitation in the bottom of collection tube, transfer the supernatant to a clean centrifuge tube before performing step 3.

3. Add Buffer RL2 (about 500  $\mu\text{L}$ ) into the supernatant (Volume of supernatant : Buffer RL2 = 1:1.6) and mix gently.

Note: the amount of Buffer RL2 added depends on the actual volume of supernatant. For instance, add 800  $\mu\text{L}$  Buffer RL2 to 500  $\mu\text{L}$  supernatant. If there is floc precipitation or the mixture solution becomes turbid, please proceed directly to step 4.

4. Transfer the mixture solution (about 700  $\mu\text{L}$ ) to RNA-Only Column (put RNA-Only Column into collection tube), centrifuge at 12,000 rpm (~13,400  $\times g$ ) for 1 minute, and discard the effluent in collection tube.

Note: If there is floc precipitation in the mixture solution, please also transfer it to RNA-Only Column.

5. Put RNA-Only Column back into the collection tube, transfer all the remaining mixture solution to it, centrifuge at 12,000 rpm (~13,400  $\times g$ ) for 1 minute, and discard the effluent in collection tube.
6. Add 500  $\mu\text{L}$  Buffer RW1 to RNA-Only Column, centrifuge at 12,000 rpm (~13,400  $\times g$ ) for 1 minute, and discard the effluent in collection tube.
7. Add 700  $\mu\text{L}$  Buffer RW2 into RNA-Only Column, centrifuge at 12,000 rpm (~13,400  $\times g$ ) for 1 minute, and discard the effluent in collection tube.
8. Repeat step 7.
9. Put RNA-Only Column back into the collection tube, centrifuge at 12,000 rpm (~13,400  $\times g$ ) for 2 minutes to remove the remaining Buffer RW2 in RNA-Only Column.
10. Transfer RNA-Only Column to a new RNase-Free centrifugal tube, add 50-200  $\mu\text{L}$  RNase-Free ddH<sub>2</sub>O which has already been heated at 65°C to the center of the membrane of RNA-Only Column. Leave it at room temperature for 2 minutes, centrifuge at 12,000 rpm (~13,400  $\times g$ ) for 1 minute, and then collect the RNA solution.

Note: The volume of RNase-Free ddH<sub>2</sub>O should not be less than 50  $\mu\text{L}$ , otherwise it might affect the elution efficiency. The RNA solution after centrifugation can be added to the membrane of RNA-Only Column again, and step 10 can be repeated to increase RNA yield.

The product RNA can be used directly in downstream experiment or stored in -80°C. Due to the careful protection of secondary RNA structure supplied by this kit, it is better to leave the product RNA in 72°C for 5-10 minutes to dissociate the secondary structure before the gel electrophoresis.