For research use only Version Number：2.0-1801

Animal Total RNA Isolation Kit

For total RNA purification from animal tissues and cells (Animal tissues ≤ 20 mg; Cells ≤ 5 ×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_2O	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 RNA extracted by the kit will not be degraded as 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)×10^6
Elution volume	50-200 μL	Maximum loading volume	850 μL

Storage

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

Note: If it is stored at a low temperature, it will crystallize easily. Please place the kit solution at room temperature (15-25℃)for a few minutes before use, or preheat it in a 37℃ water bath for 10 minutes before use to dissolve the crystals.

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 (μg)	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℃), 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 μL Buffer RL1, the recommended maximum tissue amount is 10~20mg.
- Before using the kit, please add 2-hydroxy-1-ethanethiol to Buffer RL1 (10 μL 2-hydroxy-1-ethanethiol per 1 mL Buffer RL1). Buffer RL1 can be stored at 4℃ 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 μL, 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℃) or 37℃ 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 μL Buffer RL1 and evenly grind with glass or electric homogenizer.
   Note: The amount of tissue should not exceed 20 mg, or it may cause the blocking 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 after collecting centrifugal cells (500 μL Buffer RL1 for every 1-5×10^6 cells), blow the cells repeatedly (Until aggregated cells cannot be seen).
      2) Suspension cells: add Buffer RL1 after collecting centrifugal cells (500 μL Buffer RL1 for every 1-5×10^6 cells), blow the cells repeatedly (Until aggregated cells cannot be seen).
   Note: RNA will not be contaminated by RNase in Buffer RL1. The cells or tissue can be stored at room temperature for approximately 24 hours if they are not used immediately after being lysed by Buffer RL1. Also, they can be stored at 4℃ for about 1 week, and for long time storage, please keep them in -80℃ environment. Heat them in 37℃ or leave them at room temperature before use.
2. Transfer the homogenate which has been grinded evenly to DNA-Cleaning Column (put DNA-Cleaning Column into collection tube), and centrifuge at 12,000 rmp (~13,400 ×g) for 2 minutes. Remove DNA-Cleaning Column and keep the supernatant in the collection tube.
   Note: Transfer the homogenate to a 1.5 mL centrifugal tube, centrifuge at 12,000 rpm (~13,400 ×g) for 5 minutes. If the amount of tissue is more than 20 mg or there are many lumps after grinding, please proceed directly to step 2 after the supernatant running through the column of filtration. If there is precipitation in the bottom of collection tube of DNA-Cleaning Column ,please transfer the supernatant to a clean centrifugation tube before step 3.
3. Add Buffer RL2 (about 500 μL) into the supernatant (Volume of supernatant : Buffer RL2 = 1:1.6), mixing gently.
   Note: the addition of the amount of buffer RL2 is determined by the actual volume of supernatant. For instance, add 800 μL Buffer RL2 into 500 μL 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 μL) to RNA-Only Column (put RNA-Only Column into collection tube), centrifuge at 12,000 rpm (~13,400 ×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 ×g) for 1 minute, and discard the effluent in collection tube.
6. Add 500 μL Buffer RW1 to RNA-Only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
7. Add 700 μL Buffer RW2 into RNA-Only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, 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 ×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 uL RNase-Free ddH_2O which has already been heated at 65℃ to the center of the membrane of RNA-Only Column. Leave it at room temperature for 2 minutes, centrifuge at 12,000 rmp (~13,400 ×g) for 1 minute, and then collect the RNA solution.
    Note: The volume of RNase-Free ddH_2O should not be less than 50 uL, 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℃. Due to the careful protection of secondary RNA structure supplied by this kit, it is better to leave the product RNA in 72℃ for 5-10 minutes to dissociate the secondary structure before the gel electrophoresis.

