RNApure Tissue&Cell Kit

Animal Tissue/Cell RNA Extraction Kit

Project No. R666020 (50 preps)
Storage conditions: room temperature (15-30° C)

Product content

individual parts making up a compound	R666020 50 preps
Buffer RL	35 ml
Buffer RW1	40 ml
Buffer RW2 (concentrate)	11 ml
RNase-Free Water	10 ml
Spin Columns RM with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	50

Product Introduction

This kit combines highly efficient guanidine isothiocyanate cleavage with silica matrix membrane purification for the efficient extraction of total RNA from animal cells and tissues, typically up to 30 mg of tissue or 1 x 107 cells as a starting sample. The kit also allows for the recovery of incompletely purified RNA, in vitro transcribed RNA and RNA from enzymatic reactions, allowing for the extraction and purification of high-quality RNA with molecular weights greater than 200 bases, with virtually no DNA residue. For RNA experiments that are sensitive to trace amounts of DNA, residual DNA can be removed by on-column digestion with RNase-free DNase I. The extracted RNA can be used for RNA analysis in the RNase I column. The extracted RNA can be used for RT-PCR, Nothern Blot, Dot Blot and other downstream experiments.

Self-contained reagents: β-mercaptoethanol, anhydrous ethanol (freshly opened or for RNA extraction).

Pre-experiment Preparation and Important Notes

1. To prevent RNase contamination, attention should be paid to the following aspects:
1) Use RNase-free plastics and tips to avoid cross-contamination.
(2) Glassware should be dry baked at a high temperature of 180 ℃ for 4 hours before use, plasticware can be immersed in 0.5 M NaOH for 10 minutes, rinsed thoroughly with water and autoclaved.
3) RNase-free water should be used to prepare the solution.
(4) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.
2. Avoid repeated freezing and thawing of the extracted samples, as this will affect the amount and quality of the RNA extracted.
3. Before use, please check the Buffer RL for any crystallization or precipitation, which can be re-solved by heating at 56° C. Add β-mercaptoethanol to Buffer RL before use to a final concentration of 1%. For example, add 10μl of β-mercaptoethanol to 1ml of Buffer RL. Buffer RL with β-mercaptoethanol can be stored for 1 month at room temperature.
4. Anhydrous ethanol should be added to Buffer RW2 according to the instructions on the label of the reagent bottle before first use.
5. All centrifugation steps are carried out at room temperature unless otherwise indicated and all steps are performed quickly.
6. If the downstream experiments are very sensitive to DNA, it is recommended that RNA be treated with RNase-free DNase I.

procedure

1. Sample handling
1a Tissue: Grind tissue in liquid nitrogen. Add 600 μl Buffer RL for every 20-30 mg of tissue (check for addition of β-mercaptoethanol prior to use) and 350 μl Buffer RL for tissue samples less than 20 mg. Sample volume should be no more than one-tenth of the Buffer RL volume.
1b Cells in monolayer culture: Cells are lysed or processed directly into cell suspension in culture flasks, centrifuged to obtain cell sediment, discard the supernatant, and add 600 μl Buffer RL for every 6-10 cm2 of culture area, and 350 μl Buffer RL for every <6 cm2, and blow several times to lyses them sufficiently.
1c Cell suspension: centrifuge at 12,000 rpm (~13,400×g) for 1 min and discard the supernatant to obtain the cell precipitate. Add 600 μl Buffer RL for every 5×106-1×107 cells, and 350 μl Buffer RL for less than 5×106 cells, and blow several times to fully lysate the cells.
Attention:
1) Try to get rid of the cell culture medium, which may inhibit cell lysis affecting RNA yield.
2) Try to keep the cells well suspended and well lysed, otherwise RNA yield is affected.

2. After the samples were fully lysed, they were left at room temperature for 5 minutes to allow complete separation of the protein-nucleic acid complexes.
3. Centrifuge at 12,000 rpm for 2-5 min and remove the supernatant for the next step.
4. Add 1x volume (600 μl or 350 μl) of 70% ethanol (prepared without RNase water) and mix well.
Note: The addition of ethanol may produce a precipitate that will not affect subsequent experiments.
5. Add all of the solution from step 4 to the Spin Columns RM in the collection tube. If you cannot add all of the solution to the column at once, transfer it in two batches, centrifuge the column at 12,000 rpm for 1 minute, pour out the waste liquid from the tube, and put the column back into the collection tube. Note: The maximum loading capacity of the column is 100 μg. Do not overload the column as this may affect the yield and purity of the RNA.
6. Add 700 μl of Buffer RW1 to the column, centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid from the collection tube, and return the column to the collection tube.
OPTIONAL STEP: If RNA experiments that are very sensitive to trace DNA are to be performed, replace step 6 with the following step.
1) Add 350 μl of Buffer RW1 to the column, centrifuge at 12,000 rpm for 15 seconds, discard the waste liquid, and return the column to the collection tube.
(2) Preparation of DNase I mixture: Take 52 μl of RNase-Free Water, add 8 μl of 10×Reaction Buffer and 20 μl of DNase I (1 U/μl) to it, mix well, and prepare a final volume of 80 μl of reaction solution.
Note: The above system is configured according to our product DNase I reaction system, please refer to the corresponding manual for other products.
3) Add 80 μl of prepared DNase I reaction solution directly to the adsorption column and incubate at 20-30° C for 15 minutes.
4) Add 350 μl of Buffer RW1 to the column, centrifuge at 12,000 rpm for 15 seconds, discard the waste liquid, and return the column to the collection tube.
7. Add 500 μl of Buffer RW2 to the column (check that anhydrous ethanol is added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid from the collection tube, and return the column to the collection tube.
8. Repeat step 7.
9. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.
Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
10. Place the adsorbent column in a new RNase-free centrifuge tube, add 30-50 μl of RNase-Free Water to the center of the adsorbent column overhanging the column, let it stand at room temperature for 1 minute, centrifuge it at 12,000 rpm for 1 minute, collect the RNA solution, and store the RNA at -70° C to prevent degradation.
Attention:

(1) The volume of RNase-Free Wate should not be less than 30 μl, too small a volume affects the recovery rate.
2) If you want to increase the RNA yield, repeat step 10 with 30-50 μl of fresh RNase-Free Water.
3) If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column and step 10 repeated.