Animal Tissue/Cell RNA Extraction Kit (DNase I)

Project number: R669990

Storage conditions: -20° C.

Products

individual parts making up a compound	50T
DNase I	1000U
10×Reaction Buffer	1000μl
Buffer RL	35ml
Buffer RW1	30ml
Buffer RW2 (concentrate)	11ml
RNase-Free Water	10ml
Spin Columns RM with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	50

Products

This kit combines highly efficient guanidine isothiocyanate cleavage technology 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 1x107 cells as a starting sample. The kit also allows recovery of incompletely purified RNA, in vitro transcription and RNA from enzymatic reactions. high quality RNA with molecular weights greater than 200 bases can be extracted and purified using the kit with virtually no DNA residue. If RNA experiments that are very sensitive to trace DNA are to be performed, residual DNA can be removed by on-column digestion using RNase-free DNase. The extracted RNA can be used in downstream experiments such as RT-PCR, Nothern Blot and Dot Blot.

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) RNase-free water should be used to prepare the solution.
(3) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.
2. Avoid repeated freezing and thawing of the extracted samples, otherwise it will affect the amount and quality of RNA extraction.
3. Please add β-mercaptoethanol to Buffer RL before use, 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 before first use according to the instructions on the reagent bottle label.
5. Buffer RL may be heated at 56° C to dissolve if precipitation occurs and then left at room temperature.
All centrifugation steps are performed at room temperature and all maneuvers are performed quickly.

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 before use), and 350 μl Buffer RL for tissue samples of less than 20 mg. Sample volume is not to exceed one-tenth of the Buffer RL volume.
1b Cells in monolayer culture: Lysed or processed into cell suspension directly in culture flask, centrifuged to obtain cell precipitate, discarded the supernatant, added 600μl Buffer RL for every 6-10 cm2 of culture area, 350μl Buffer RL for less than 6cm2, and blown several times repeatedly to make the cells lysed 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 repeatedly to fully lysate.
Note: 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 sample is fully lysed, leave it at room temperature for 5 minutes to allow complete separation of the protein-nucleic acid complex.
3. Centrifuge at 12,000 rpm for 2-5 min and remove the supernatant for the following operations.
4. Add 1x volume (600μl or 350μl) of 70% ethanol (prepared without RNase water) to the solution obtained in step 3 and mix well.
Note: The addition of ethanol may produce a precipitate that will not affect subsequent experiments.
5. Add all of the solution obtained in the previous step 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 passes, centrifuge at 12,000 rpm for 1 minute, and discard the waste solution. Place the column back into the collection tube.

Note: The maximum loading capacity of the adsorption column is 100μg, do not overload as this will affect the yield and purity of the RNA.

6. Add 350 μl Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid and put the adsorbent column back into the collection tube.

7. 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.

8. Add 80μl of DNase I mixture directly to the adsorption column and incubate at 20-30° C for 15 minutes.

9. Add 200 μl Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid and put the adsorbent column back into the collection tube.

10. Add 500μl 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 in the collection tube, and put the column back into the collection tube.

11. Repeat step 10.

12. 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 a few minutes to thoroughly dry the anhydrous ethanol in the adsorption column.

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.).

13. Transfer the adsorbent column into a new centrifuge tube, add 30-50 μl of RNase-Free Water to the middle of the adsorbent membrane, leave it at room temperature for 1 min, centrifuge at 12,000 rpm for 1 min, collect the RNA solution, and store the RNA at -70° C to prevent degradation.

Note: 1) The volume of RNase-Free Wate should not be less than 30 μl, too small volume affects the recovery rate.

2) If you want to increase the RNA yield, repeat step 13 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 13 repeated.