Ultrapure RNA Extraction Kit

Project number: U670000

Storage conditions: 2-8° C, protected from light.

Products

individual parts making up a compound	50T	200T
TRIzon Reagent	60ml	2 x 110mL
TRIzon PaI ™	10ml	2×20mL
Buffer RW1	40ml	160ml
Buffer RW2 (concentrate)	11ml	50ml
RNase-Free Water	10ml	50ml
Spin Columns RM with Collection Tubes	50	200
RNase-Free Centrifuge Tubes (1.5 mL)	50	200

Products

This kit is based on TRIzon improved column total RNA extraction kit, the lysis solution fully lysed and homogenized samples, using a unique silicon matrix membrane adsorption technology, through the centrifugal adsorption column in the high salt state in the high efficiency and specific binding solution RNA, while maximizing the effective removal of proteins, inorganic salts, ions, and organic impurities, etc.; can be used to rapidly extract total RNA from samples such as animal tissues, plant materials, various microorganisms and cultured cells, etc. Each time, 30-50 mg of tissue or 5 × 10^6 cells can be processed, and several different samples can be processed simultaneously. Total RNA can be rapidly extracted from animal tissues, plant materials, various microorganisms and cultured cells, etc. It can process 30-50 mg of tissues or 5×10^6 cells each time, and can process several different samples at the same time. The extracted RNA can be directly applied to RT-PCR, Northern Blot, Dot Blot, in vitro translation and other experiments.

Product Features

- High purity: Maximum removal of protein and other impurities, the extracted RNA can be directly used in various downstream experiments.
- High extraction volume: Unique lysate formulation, fully lysed cells or tissues, RNA extraction volume as much as 100 μg.

- Fast: fewer steps, easier to operate, saves time.
- Strong compatibility: Suitable for the extraction of RNA from a wide range of animal and plant tissues and cells.

Self-contained reagents: 70% ethanol (prepared without RNase water), anhydrous ethanol.

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-roasted at 180° C for 4 hours before use, and plasticware can be soaked in 0.5M 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. Samples should avoid repeated freezing and thawing, otherwise it will affect the rate and quality of RNA extraction.
3. If any precipitation is found in TRIzon Reagent before use, it can be dissolved by placing it in a 56℃ water bath for a few minutes.
4. Anhydrous ethanol should be added to Buffer RW2 according to the label instructions of the reagent bottle before first use.
5. All centrifugation steps are carried out at room temperature if not otherwise indicated, and all steps are performed quickly.
6. If the downstream experiments are very sensitive to DNA, it is recommended to treat the RNA with RNase-free DNase I.

Usage

1. Sample handling
1a. Plant tissue: Take fresh plant tissue and grind it well in liquid nitrogen or cut the plant tissue and grind it quickly directly in TRIzon Reagent, add 1mL of TRIzon Reagent for every 30-50mg of tissue and mix well.
Note: The sample volume should not exceed 10% of the TRIzon Reagent volume.
1b. Animal tissues: Take fresh or -70℃ frozen animal tissues and cut as much as possible, add 1mL TRIzon Reagent per 30-50mg of tissue, homogenizer for homogenization. Or add 1mL of TRIzon Reagent after grinding in liquid nitrogen and mixing.
Note: The sample volume should generally not exceed 10% of the TRIzon Reagent volume.
1c. Monolayer culture cells: Aspirate off the culture solution, add appropriate amount of TRIzon Reagent (1mL TRIzon Reagent per 10cm² area) directly into the culture plate, and blow repeatedly with a sampler to make the cells lysed. Alternatively, after trypsin treatment, transfer the cell solution to an RNase-Free centrifuge tube, centrifuge at 300×g for 5 min, collect the cell precipitate, carefully aspirate all the supernatant, and add 1mL of TRIzon Reagent and mix well.
Attention:

1) Do not collect more than 1 x 107 cells.
2) The amount of TRIzon Reagent added is determined by the plate area, not by the number of cells. If the amount of TRIzon Reagent is not sufficient, DNA contamination in the extracted RNA may result.
3) Be sure to remove the cell culture fluid when collecting the cells, otherwise it will lead to incomplete lysis, resulting in a lower yield of RNA.
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1d. cell suspension: collect cells by centrifugation. Add 1mL TRIzon Reagent per 5×10^{6}-1×10^{7} animal, plant and yeast cells or per 10^{7} bacterial cells.
Attention:
1) Do not wash the cells before adding TRIzon Reagent to avoid RNA degradation.
2) Some yeast and bacterial cells may require homogenizer or liquid nitrogen grinding treatment.
1e.Blood Treatment: Take fresh blood directly, add 3 times the volume of TRIzon Reagent (0.25mL whole blood to 0.75mL TRIzon Reagent is recommended), and mix with thorough shaking.
1f. Optional step: For samples with a high content of proteins, fats, polysaccharides, or extracellular material, such as muscle tissue, adipose tissue, or tubers from plants, centrifugation at 4° C, 12,000 rpm (~13,400 x g) for 10 minutes after homogenization may be performed to remove insoluble material, at which time the precipitate contains extracellular material, polysaccharides, and high-molecular-weight DNA, whereas RNA is present in the supernatant.
2. Blow the sample several times repeatedly after adding TRIzon Reagent to the sample to fully lyse the sample. Leave at room temperature for 5 minutes to allow complete separation of the protein-nucleic acid complex.
3. Add 200 μL of TRIzon PaI ™ per 1 mL of TRIzon Reagent, cap the tube, shake vigorously for 15 seconds, and let stand at room temperature for 2 minutes.
4.4° C 12,000 rpm (~13,400 × g) centrifugation for 10 min, at which point the sample is divided into three layers: red organic phase, middle layer and upper colorless aqueous phase, with the RNA predominantly in the upper aqueous phase, which is transferred to a new RNase- Free centrifuge tube (self-provided).
5. Add an equal volume of 70% ethanol (prepared without RNase water) to the resulting aqueous phase solution, invert and mix well.
6. Add all of the solution from the previous step to the Spin Columns RM in the collection tube. If the solution cannot be added all at once, it can be transferred in several times. centrifuge at 12,000 rpm for 20 seconds, pour out the waste liquid from the collection tube, and put the column back into the collection tube.
7. Add 700 μL of Buffer RW1 to the adsorbent column and centrifuge at 12,000 rpm for 20 s. Pour off the waste liquid in the collection tube and put the adsorbent column back into the collection tube.
8. Add 500 μL of Buffer RW2 to the adsorbent column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 20 seconds, pour off the

waste liquid in the collection tube, and put the adsorbent column back into the collection tube.

9. Repeat step 8.

10. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the column at room temperature for a few minutes and dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

11. Place the adsorbent column in a new RNase-free centrifuge tube, add 30-50 μL of RNase-Free Water to the middle of the adsorbent column, leave it at room temperature for 1 min, centrifuge it at 12,000 rpm for 1 min, 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 11 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 11 repeated.