Ultrapure RNA Kit (DNase I)

Ultrapure RNA Extraction Kit (DNase I)

Item No. U665516 (50 preps)
Storage conditions: DNase I and 10×Reaction Buffer at -20℃, TRIzon PaI™ and TRIzon Reagent.
Store at 2-8° C away from light, other components at room temperature (15-30° C).

Product content

individual parts making up a compound	U665516 50 preps
DNase I	1000 U
10×Reaction Buffer	1000 μl
TRIzon Reagent	60 ml
TRIzon PaI™	10 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 is an improved total RNA extraction kit based on TRIzon, which can extract total RNA from animal tissues, plant materials, various microorganisms and cultured cells, etc. Firstly, the lysate lyses and homogenizes the samples, and then in the unique high salt state, the RNA binds to the silica matrix membrane, which reduces the protein contamination to a great extent and removes organic solvent contamination effectively. The RNA obtained is of better purity and quality. This product can rapidly extract total RNA from various cells or tissues, and can process 30-50 mg of tissue or 5×10^{6} cells each time, and can process several different samples at the same time. For RNA experiments that are very sensitive to trace DNA, the residual DNA can be removed by RNase-free DNase digestion on the column, and the extracted RNA can be directly applied to RT-PCR, Northern Blot, Dot Blot, in vitro translation and other experiments.

Pre-experiment Preparation and Important Notes

1. To prevent RNase contamination, attention should be paid to the following aspects:
1) Plastic products and tips of RNase 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 samples, as this may affect the rate and quality of RNA extraction.
3. If precipitation is found in the TRIzon Reagent before use, it can be dissolved by placing it in a water bath at 56° C 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 performed at room temperature unless otherwise indicated, and all steps are performed quickly.

Using the method

1. Sample processing
1a.Tissue: 30-50 mg of tissue is fully ground in liquid nitrogen and then added to 1 mL of TRIzon Reagent, or 1 mL of TRIzon Reagent is added to the tissue sample and homogenized.
Note: The sample volume should not exceed 10% of the TRIzon Reagent volume.
2a.Monolayer culture cells: Aspirate off the culture solution and add appropriate amount , 1 mL TRIzon Reagent per 10 cm^2.
3a. Cell suspension: collect cells by centrifugation. Add 1 mL TRIzon Reagent per 5 x 10^6 cells.
2. Blow several times after adding TRIzon Reagent to fully lyse the sample. Allow to stand at room temperature for 5 minutes to allow complete separation of protein-nucleic acid complexes. 3.
3. Add 200 μL of TRIzon PaI ™ per 1 mL of TRIzon Reagent, cap the tube, shake vigorously for 15 seconds, and allow to stand at room temperature for 2 minutes.
4. Centrifuge at 12,000 rpm (~13,400 x g) for 10 minutes at 4° C. At this point, the sample is divided into three layers: a red organic phase, an intermediate layer, and an upper colorless aqueous phase, with the RNA predominantly in the upper aqueous phase; transfer the upper aqueous phase 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 obtained in the previous step to the Spin Columns RM that have been loaded into 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 off the waste liquid from the collection tube, and put the column back into the collection tube.

7. Add 350 μL of Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 20 seconds, pour off the waste liquid from the collection tube, and return the adsorbent column to the collection tube.

8. Prepare 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), mix well, and prepare a final volume of 80 μL of reaction solution.

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

10. Add 350 μL of Buffer RW1 to the column, centrifuge at 12,000 rpm for 1 minute, discard the waste solution, and return the column to the collection tube.

11. Add 500 μL of Buffer RW2 to the adsorbent column (check that anhydrous ethanol has been added prior to use), centrifuge at 12,000 rpm for 20 seconds, pour off the waste liquid from the collection tube, and place the adsorbent column back into the collection tube.

12. Repeat step 11.

13. 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 several minutes and allow 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.).

14. 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, allow to stand at room temperature for 1 minute, centrifuge 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.

2) If you want to increase the RNA yield, repeat step 14 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 14 repeated.