Column type ultrapure animal RNA small extraction kit

CAT: R2010

Size: 50T

Storage: RT, 12 months. Among them, RNase-free DNase can be stored at 4°C .

Product Introduction：

This product utilizes the principle that RNA can be efficiently combined with silicon matrix materials in a specific buffer system, and uses a silica gel membrane centrifugal adsorption column, which is suitable for extracting total RNA from cultured cells and animal tissues, and can effectively extract RNA with a molecular weight greater than 200 nt. During the extraction process, there is no need to use phenol, chloroform, etc., and DNase column processing is used to completely remove genomic DNA residues. The extracted RNA samples are tested by PCR to contain no genomic DNA, and contain very little protein and other impurities. This product is simple and fast to operate, and the extracted RNA is of high purity. It can be directly used in various molecular biology experiments such as RT-PCR, Northern blot, and cDNA library construction.

Product Composition：

Component	50T	Notes
Column balance solution	30 mL	
Proteinase K	0.6 mL	
Tissue/cell lysate	40 mL	
Washing buffer 1（WB1）	40 mL	
Washing buffer 2（WB2）	12 mL	Please add according to the bottle label before first use Absolute ethanol (48 mL)
RNase-free DNase（2000 U）	1 bottle	Store at 4°C
Membrane reaction solution	4 mL	
RNase-free ddH_2O（Tube）	1 mL	
RNase-free ddH_2O（bottled）	15 mL	
RNase-free syringe	1	
Adsorption column	50	
2 mL Collection tube	50	
1.5 mL RNase-free Centrifuge tube	50	

Experiment preparation

Users need to prepare their own reagents: β-mercaptoethanol

1. Preparation of RNase-free DNase mother solution: Use a 1 mL RNase-free syringe to draw 550 μL of RNase-free ddH2O into a glass bottle containing RNase-free DNase (2000 U) dry powder, mix gently, and then aliquot Store at -20°C (can be stored for 9 months).

Note: The RNase-free DNase mother liquor thawed from -20°C is stored at 4°C (can be stored for 6 weeks), do not freeze again.

2. The tissue/cell lysate may form a precipitate, please heat it at 60 ℃ to dissolve it, and then use it after returning to room temperature. Please determine the amount of "tissue/cell lysate" required according to the number of samples taken, transfer to a new centrifuge tube, and add 1% β-mercaptoethanol of the "tissue/cell lysate" volume taken. It is recommended that the lysate is prepared now. If the prepared lysate is not used up, it can be stored at 4°C for 1 month.

3. Before the first use, add 48 mL of absolute ethanol to theWashing buffer 2 (WB2) according to the bottle label, and mark it.

Protocol (For reference only):

Cultured cells

1. Column equilibration: Put the adsorption column into a 2 mL collection tube, add 500 μL of column equilibrium solution to the adsorption column, centrifuge at 12000 rpm for 2 minutes, and discard the waste liquid in the collection tube. Please use the column processed that day.

2. Cell lysis:

1) Adherent cells: aspirate and discard the culture medium thoroughly, add 600 μL of tissue/cell lysate per 6-10 cm^2 area (please add β-mercaptoethanol freshly before use), pipette 3-5 times to make the cells Cracked.

2) Cell suspension: Collect cells by centrifugation at 500 g, thoroughly aspirate the culture medium, add 600 μL of tissue/cell lysate for every 5×10^6-1×10^7 cells (please add β-mercaptoethanol freshly before use), and pipette Pipette 3-5 times to lyse the cells.

3. Centrifuge at 12000 rpm for 2 minutes, carefully transfer the supernatant to a new 1.5 mL centrifuge tube, and try to avoid touching the cell debris in the tube.

4. Slowly add 0.5 times the filtrate volume of absolute ethanol, mix well (precipitation may occur at this time), transfer the obtained solution and precipitate to the adsorption column (the adsorption column is placed in the collection tube), and centrifuge at 12000 rpm for 30 s , Discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

5. Add 350 μL of Washing buffer 1 (WB1) to the adsorption column, centrifuge at 12000 rpm for 30 s, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

6. Preparation of RNase-free DNase working solution: Take 10 μL of RNase-free DNase mother solution into a new RNase-free centrifuge tube, add 70 μL of membrane reaction solution and mix gently.

7. Add 80 μL of RNase-free DNase working solution to the center of the adsorption column and leave it at room temperature for 15 minutes.

8. Add 350 μL Washing buffer 1 (WB1) to the adsorption column, centrifuge at 12000 rpm for 30 s, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

9. Add 500 μL Washing buffer 2 (WB2) to the adsorption column (please check whether ethanol has been added before use), centrifuge at 12000 rpm for 30 s, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube .

10. Repeat step 9 once.

11. Centrifuge at 12000 rpm for 3 minutes at room temperature. This step is very important, otherwise the residual ethanol (component in WB2) will affect the use of RNA.

12. Put the adsorption column into a new 1.5 mL sterile centrifuge tube, add 50-100 μL RNase-free ddH_2O to the center of the adsorption column, leave it at room temperature for 1 min, and centrifuge at 12000 rpm for 1 min to obtain the RNA solution. The resulting RNA solution should be used immediately or stored at -80°C after appropriate aliquots.

Animal tissue

1. Column equilibration: Put the adsorption column into a 2 mL collection tube, add 500 μL of column equilibrium solution to the adsorption column, centrifuge at 12000 rpm for 2 minutes, and discard the waste liquid in the collection tube. Please use the column processed that day.

2. Tissue homogenization and lysis:

1) Liquid nitrogen grinding: Take 300 μL of tissue/cell lysate (check whether β-mercaptoethanol has been added before use) and add it to a 1.5 mL centrifuge tube. After the tissue sample is ground with liquid nitrogen, add the ground sample (10-20 mg) to the above 1.5 mL centrifuge tube containing 300 μL tissue/cell lysate, shake vigorously and mix until there is no obvious precipitation in the lysate .

2) Electric homogenizer: Take 300 μL of tissue/cell lysate (please check whether β-mercaptoethanol has been added before use) and add it to a 1.5 mL centrifuge tube. Take 10-20 mg of tissue and add it to the above-mentioned tissue containing 300 μL In the centrifuge tube of the cell lysate, use an electric homogenizer to grind thoroughly.

3) Add 590 μL RNase-free ddH_2O and 10 μL Proteinase K to the above-mentioned tissue lysis mixture, and incubate at 56°C for 10-20 min after mixing.

Note: The tissue sample should not exceed 20 mg, otherwise the yield and purity of RNA extracted will decrease.

3. Centrifuge at 12000 rpm for 2 minutes, carefully transfer the supernatant in the tube to a new 1.5 mL centrifuge tube, and try to avoid touching the cell debris in the tube.

4. Slowly add 0.5 times the supernatant volume of absolute ethanol, mix well (precipitation may occur at this time), transfer the obtained solution and precipitate to the adsorption column (the adsorption column is placed in the collection tube), and centrifuge at 12000 rpm 30 s, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

5. Add 350 μL of Washing buffer 1 (WB1) to the adsorption column, centrifuge at 12000 rpm for 30 s, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

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Notes：

1. This product is for scientific research use only. Do not use it for medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.
2. For your safety and health, please wear lab coats and wear disposable gloves and masks.