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RNApure Tissue&Cell Kit （DNase I）

Catalog Number: CW0560S (50 preps)

Storage Condition: Room temperature (15-30°C)

Kit Components:

Component	CW0560S (50 preps)
DNAse I	1000U
10x reaction buffer	1ml
Buffer RL	35 ml
Buffer RW1	30 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:

The kit uses spin columns which efficiently and specifically bind with nucleic acid and a unique buffer system, to extract total RNA rapidly from animal tissues (up to 30 mg) or cultured cells (up to $1x10^7$). The extracted total RNA has high purity and is free of protein and other contaminations. The extracted RNA can be directly used in downstream experiments such as RT-PCR, real-time RT-PCR, Northern Blot, and in vitro translation etc.

Not included in the kit:

β-mercaptoethanol; 100% ethanol

Precautions:

1. To prevent RNase contamination, the following aspects should be noted:
(1) Use RNase-free plastic products and tips to avoid cross-contamination.
(2) Glassware should be dry heated at 180°C for 4 hours before use. Plastic containers can be immersed in 0.5 M NaOH for 10 minutes, then rinse thoroughly with water and autoclave.
(3) Use RNase-free water to prepare the solutions.
(4) Operators should wear disposable masks and gloves, and change gloves frequently during the experiment.
2. Avoid repeated freezing and thawing of the samples, otherwise it affects the yield and quality of RNA extraction.
3. Check if there is precipitation in Buffer RL before use. If yes, put it in a 56°C water bath for re-dissolve.
4. Add β-mercaptoethanol to Buffer RL before use, and the final concentration is 1% (add 10 μl to 1 ml Buffer RL). The Buffer RL added with β-mercaptoethanol can be stored at room temperature for one month.
5. 100% ethanol should be added to Buffer RW2 before the first use according to instructions on the reagent bottle label. Prepare 70% ethanol.
6. All centrifuge steps are performed at room temperature unless otherwise specified, and all manipulation steps should be performed quickly.

Protocol:

1. **For cells:** Harvest cells according to step **1a or 1b**. **1a.** Cells grown in suspension (less than 1 x 10^7 cells): Pellet the appropriate number of cells by centrifuging for 5 min at 300 x g in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration and proceed to step **1c. 1b.** Cells grown in a monolayer (less than 1 x 10^7 cells): Cells can be either lysed

directly in the cell-culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell-culture flasks should always be trypsinized.

1c. Add 350 µl (for cells less than $5x10^6$) or 600 µl (for cells between $5x10^6$-$1x10^7$) Buffer RL (check if β-mercaptoethanol is added before use), Vortex or pipet to mix. Homogenize by vortexing for 1 min.

For Animal Tissues

Disrupt the less than 20 mg tissue and homogenize the lysate in 350 µl Buffer RL (check if β-mercaptoethanol is added before use), with a rotor–stator homogenizer or other methods. Add 600 µl Buffer RL for every 20-30 mg tissue.

2. After the sample was fully lysed, leave the samples at room temperature for 5 minutes.
3. Centrifuge at 12,000 rpm for 2-5 minutes, transfer the supernatant to a new 1.5 ml tube.
4. Add 1 volume (350 or 600 µl) of 70% ethanol to the solution obtained from above step and mix well (precipitation may occur now).
5. Transfer less than 700 µl solution and precipitation to Spin Columns RM with collection tubes, and centrifuge at 12,000 rpm for 1 minute. Discard the waste in the collection tubes and put the columns back to the collection tubes. If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same spin column RM. Discard the flow-through after each centrifugation.
6. Add 350 µl of Buffer RW1 to the column, then centrifuge at 12,000 rpm for 1 minute. Discard the waste liquid in the collection tube and put the column back into the collection tube.
7. Make DNase I mixture: Take out 52 µl of RNase-Free Water, then add 8 µl of 10x Reaction Buffer and 20 µl of DNase I . Mix well to make 80 µl of reaction solution.
8. Add 80 µl of prepared DNase I reaction solution to the column directly. Incubate at 20-30°C for 15 minutes.
9. Add 200 µl of Buffer RW1 to the column, then centrifuge at 12,000 rpm for 1 minute. Discard the waste liquid in the collection tube and put the column back into the collection tube.
10. Add 500 µl of Buffer RW2 to the column (check whether 100% ethanol has been added before use), then centrifuge at 12,000 rpm for 1 minute. Discard the waste liquid from the collection tube and place the column back into the collection tube.
11. Repeat step 10.
12. Centrifuge at 12,000 rpm for 2 minutes, then discard the waste liquid in the collection tube. Leave the column at room temperature for several minutes to dry it thoroughly.

Note: The purpose of this step is to remove the residual ethanol in the column, and the residual of ethanol will affect the subsequent enzymatic reaction (enzyme digestion, PCR, etc.)

13. Place the column in a new RNase-free collection tube and add 30-50 µl of RNase-Free water to the middle of the membrane; Leave the column at room temperature for 1 minute, then centrifuge at 12,000 rpm for 1 minutes, and the RNA is collected into a centrifuge tube. Store the RNA at -70°C to prevent degradation.

    Note:

    1) The volume of RNase-Free Water should not be less than 30 µl, because too small volume will affect the recovery rate.
    2) To increase the yield of RNA, repeat step 13.
    3) To increase the concentration of RNA, the RNA solution can be added back to the column and repeat step 13.