Tissue/Cell RNA Extraction Kit (gDNA Removal Column)

Code No.：NG3001S 50T

Contents	storage	50 T
Buffer RB	RT	30 ml
Buffer RE	RT	40 ml
Buffer RW	RT	10 ml Add absolute ethanol before use
70% ethanol	RT	9 ml Add absolute ethanol before use
RNase-free H_2O	RT	5 ml
Spin Columns RA/ Collection tubes	RT	50
Spin Columns gDNA/ Collection tubes	RT	50

Important Note：

All solutions should be clear. If the ambient temperature is low, the solutions may form precipitates and should not be used directly. In such cases, they be heated in a 37 ℃ water bath for a few minutes to restore clarity. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure the reagents to air, the caps of each solution should be tightly closed immediately after use.

Introduction：

This product utilizes the latest genomic DNA removal column technology to ensure the effective removal of gDNA The resulting RNA does not require DNase digestion and can directly used for experiments such as reverse transcription PCR and quantitative real-time PCR. A unique lysis buffer rapidly lyses cells and inactivates cellular RNases, after which the lysate is through a genomic DNA removal column, where genomic DNA is removed and RNA passes through the filter. After the filtered RNA is adjusted for binding conditions with ethanol, it is selectively adsorbed onto silica matrix membrane in the spin column under high-chaotropic salt conditions. Then, through a series of rapid wash-and-centrifugation steps, impurities such as cellular and proteins are removed. Finally, low-salt RNase-free water elutes the purified RNA from the silica matrix membrane.

Safety Information:

1. All centrifugation steps were performed at room temperature unless otherwise specified.
2. Lysis buffer RB and RE contain irritating and harmful compounds. Wear latex gloves during operation to avoid contact with skin, eyes and clothing. If contact with skin or eyes occurs, rinse with plenty of water or saline.

Operation Procedure:

Tip: Before the first use, please add absolute alcohol in the buffer RW and 70% ethanol, mark it.

1. Preparing samples.

A. Tissues:

Grind the tissue into powder. Add 350 μl (<20 mg) or 600 μl RB (20-30 mg).

Note: If there are too many insoluble fragments after grinding and homogenization, the lysate after homogenization can be centrifuged at 12,000 rpm for3 minutes to precipitate potentially difficult-to-lyse fragments or insoluble matter. Add the supernatant to the DNA removal column (place the removal column in a collection tube).

B. Adherent Cells :

Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel. Add RB (See Appendix I) directly to the cells. Pipette the lysate up and down several times until the solution becomes clear.

C. Suspension Cells:

Harvest cells by centrifugation (12,000 rpm for 10 sec) and remove culture medium completely. Add 350 µl RB (<5x10^6) or 600 µl RB per 5-10 × 10^6 cells. Pipette the lysate up and down several times until the solution becomes clear.

2. Add the solution from the previous step to Spin Columns gDNA, 12,000 rpm for 1 min or more time until all the liquid has passed through the membrane, collect the filtrate into a new tube.
3. Add 1 volume ethanol invert to mix (a precipitate may form at time). Transfer the resulting solution and any possible precipitate into a adsorption column RA.
4. Centrifuge for 30 s at 12,000 rpm. Discard the flow-through and set the Spin Column RA back into the Collection Tube.
5. Add 500 µl of Buffer RE to the Spin Column and centrifuge for 30 sec, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
6. Add 500 µl of Buffer RW to the Spin Column and centrifuge for 30 sec, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
7. Repeat step 6.
8. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
9. Add 30-80 µl of RNase-free H_2O (no less than 30 µl) to the center of the membrane, let the column stand for 2 min, then centrifuge for 1 min at 12,000 rpm. Stored at -70 ℃.

Appendix I

	base area(cm^2)	**culture medium volume(ml)**	**Cell**
24-well culture plate	2	1.0	5 ×10^5
6-well culture plate	9.6	2.5	2.5 ×10^6
3.5 cm culture plate	8	3.0	2.0 ×10^6
6 cm culture plate	21	5.0	5.2 ×10^6
25 cm culture flask	25	5.0	5.2 ×10^6
100 ml culture flask	33	10.0	7 ×10^6

Note: Generally, add 350 µl of lysis/binding buffer RB to a 3.5 cm diameter culture dish or smaller culture vessel, and 600 µl of lysis/binding buffer RB to a 6 cm diameter culture dish or larger culture vessel. The maximum processing capacity does not exceed 10^7.