NGzol plus Rapid Total RNA Extraction Kit

Code No.： NG325S 50 T

NG325M 100 T

Contents	storage	50 T	100 T
Buffer RL	4 ℃	50 ml	100 ml
RNA Extraction buffer	RT	10 ml	20 ml
Buffer RE	RT	25 ml	50 ml
Buffer RW	RT	10 ml	25 ml
		Add absolute alcohol before use	
Spin Columns RA	RT	50	100
RNase-free H_2O	RT	10 ml	20 ml
Collection tubes（2 ml）	RT	50	100

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. Improper storage at low temperatures (4 ℃ or -20 ℃) can precipitation in the solutions and affect the performance, therefore both transportation and storage should be carried out at room temperature (15 ℃ – 25 ℃). Buffer VLS can be transported at room temperature and should be stored at 4 ℃ away from light upon receipt. 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：

The improved isothiocyanate guanidine/phenol one-step method (NGzol method) lyses cells and inactivates RNases. After adding the Extraction buffer, the solution separates into a colorless aqueous phase and a pink organic phase, with the RNA present in the aqueous phase. The RNA in the aqueous phase is collected and passed a column, where the silica membrane spin column specifically adsorbs the RNA from the aqueous phase. Then, through a series of rapid wash-centrifugation steps, the protein solution and wash buffer remove impurities such as cell metabolites and proteins. Finally, low-salt RNase-free water elutes the pure RNA from the silica matrix membrane.

Safety Information:

1. All centrifugation steps were performed at room temperature unless otherwise specified.
2. Lysis buffer RL 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 mark it.

1. Preparing samples.

A. Tissues:

Add liquid nitrogen in tissues and grind them thoroughly to power. Add 1 ml RL for per 50–100 mg sample. Usually, the volume of tissue sample should not exceed 10% of the volume of RL.

B. Adherent Cells :

Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel. Add 1 ml RL directly to the cells in the culture dish per 10 cm2 of culture dish surface area. Pipette the lysate up and down several times until the solution becomes clear.

C. Suspension Cells:

Harvest cells by centrifugation and remove culture medium. Add 1 ml of RL Reagent per 5-10 × 106 cells from animal or plant. Do not wash cells before addition of RL to avoid increased chance of mRNA degradation.

2. The homogenate sample was subjected to severe shaking and left at room temperature for 5 min to allow complete dissociation of the nucleosome.

Optional step: Centrifuge the sample at 12,000 rpm for 10 min at 4 ℃. Transfer the supernatant to a fresh micro-centrifuge tube.

Note: When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional centrifugation may be required to remove insoluble material from the samples. RNA remains in the upper aqueous phase after centrifugation.

3. Add 200 μl of RNA Extraction buffer. Vortex for 15 sec. Incubate for 2-3 min at room temperature. Centrifuge at 12,000 rpm for 10-15 min at 4 ℃. The mixture separates into three phase, a lower yellow phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Pipette the aqueous phase (about 600 μl) out into a new tube.

4. Add 1/2 volume ethanol, invert to mix (a precipitate may form at time). Transfer the resulting solution and any possible precipitate into a adsorption column RA (the adsorption column is placed inside a collection tube).

5. Centrifuge for 30 s at 12,000 rpm. Discard the flow-through and set the Spin Column RA back into the Collection Tube.

6. 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.

7. 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.

8. Repeat step7.

9. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.

10. Add 15-35 μl of RNase-free H_2O 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 ℃.