NGZOL total RNA isolation kit

Code No.： NG304S 50 T

NG304M 100 T

Contents	storage	50 T	100 T
Buffer RL	4 ℃	50 ml	100 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：

Buffer RL contains phenol, which is toxic and corrosive. It can lead to poisoning, Burns, and other bodily injuries if touched. When using this product should wear protective articles, such as protective clothing, gloves, eye masks and so on. Please flush immediately with a large amount of water and proceed to hospital for treatment if careless contact.

Introduction：

Buffer RL is a wide range use reagent for total RNA extraction. The color of reagent is bright, the operation is fast and convenient. Reagent can be used to extract total RNA from animal tissues, plant materials, various microorganisms, cultured cells and so on. No matter a small amount of tissues (50-100 mg) and cells (5×10^6) or a large amount of tissues (≥1 g) and cells ($>10^7$), all have good extraction results by using this method. Buffer RL allows the adsorption of RNA onto silica membrane, then the impurities were removed by Buffer RE and RW, finally, RNA was eluted from the silica matrix membrane by RNase-free H_2O. It can be used in in RT-PCR, Real-time RT-PCR, Northern Blot, Dot Blot, in vitro translation, etc.

Safety Information:

1.Samples homogenized with RL can be placed at -70 ℃ for more than one month without immediate addition of chloroform.

2.Required reagents: chloroform and 75% ethanol (prepared with DEPC-treated water).

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 cm^2 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 $\times 10^6$ cells from animal or plant. Do not wash cells before addition of RL to avoid increased chance of mRNA degradation.

D. Blood:

RLS is recommended for use with whole blood or Liquid samples. RLS is used for Liquid Sample. Add 0.75 ml RLS for per 250 µl sample.

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 chloroform. Vortex for 15 sec. Incubate for 2-3 min at room temperature.
4. 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.
5. Add anhydrous ethanol of half volume of aqueous phase, fully mixed with immediately turn up and down. Transfer the mixture into Spin Columns RA (put in a Collection Tube). 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 600 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 50-80 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 ℃. Note: The volume of RNase-free H_2O should not be less than 30 µl, otherwise it may affect recovery efficiency.