Rapid RNA Extraction Kit for Paraffin-Embedded Tissues

Code No.： NG311S 50 T

NG311M 100 T

Contents	storage	50 T	100 T
Buffer ML	4 ℃	50 ml	100 ml
Buffer RB	RT	15 ml	30 ml
Buffer RE	RT	25 ml	50 ml
Buffer RW	RT	15 ml	25 ml
		Add absolute alcohol before use	
70% ethanol	RT	9 ml	18 ml
		Add absolute alcohol before use	
RNase-free H_2O	RT	10 ml	20 ml
Proteinase K(20mg/ml)	-20 ℃	20 mg	20 mg*2
Spin Columns RD	RT	50	100
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 ℃). 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：

An improved guanidinium isothiocyanate/phenol one-step method is used to lyse cells and inactivate RNases, then total RNA is selectively onto a silica matrix membrane in a spin column under high chaotropic salt conditions. Subsequently, through a series of rapid wash-spin steps, protein-removing solution and wash buffer remove such as cell metabolites and proteins. 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 ML, 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.
3. The unique ML lysis buffer formulation can effectively eliminate genomic contamination.
4. Multiple rinsing steps to remove proteins result in higher RNA purity.
5. Good repeatability.

Operation Procedure:

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

1. Cut 5-7 sections of 5-10 μm thickness from the paraffin-embedded tissue and place them into a 1.5 centrifuge tube.
2. Add 1 ml of xylene and vortex for 10 seconds.
3. Centrifuge at 12,000 rpm for 2 min at room temperature. Remove the supernatant, taking care not to remove the pellet.
4. Add 1 ml of ethanol, vortex to mix, and centrifuge at 12,000 rpm for 2 min at room temperature.
5. Remove the supernatant, taking care not to remove the precipitate. Air-dry at room temperature for 10 min or until the residual ethanol has completely evaporated.
6. Add 240 μl of solution RB and 10 μl of Proteinase K solution, and vortex to mix. Incubate at 55 ℃ for 15 min, then at 80 ℃ for 15 min.
7. Add 0.75 ml of buffer ML and pipette the liquid sample up and down several times with a micropipette to help lyse the cells in the sample. incubate at RT for 2 min.
8. Add 200 μl of chloroform. 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 phases, 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.
9. Add 1 volume of 70% ethanol (please check if anhydrous ethanol has been added first!), invert to mix (a precipitate may form at time). Transfer the resulting solution and any possible precipitate into a micro-adsorption column RD (the adsorption column is placed inside a collection tube).
10. Centrifuge for 30 s at 12,000 rpm. Discard the flow-through and set the Spin Column RD back into the Collection Tube.
11. 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.
12. 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.
13. Repeat step7.
14. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
15. 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 ℃.