Tissue RNA PrepMate

I. Description

This kit is designed for extraction of total RNA from tissue, cultured cell based on the Modified guanidinium salt-lysis method. Extracted total RNA from part of tissue is contaminated by genomic DNA, it is important to treat them using RNase-free DNase. Northern analysis, dot blot hybridization, poly A selection, According to the quality of total RNA used in experiments, high quality of poly(A) RNA and high-sensitivity hybridization pattern may be obtained not only for RNase protection assay, but also for molecular cloning or RT-PCR etc.

The amount of extracted total RNA depends on tissue types and cell line, refer to **Yield and purity of total RNA,** determine the start volume of samples supposed to be extracted.

II. Kit Components

K-3080 Tissue RNA PrepMate ™

Reagents	
Lysis Buffer	100 ml
Phenol:Chloroform(5:1)	100 ml
RNA Hydration Solution	10 ml
User's Guide	
Store all reagents at 4 °C	

III. Protocols

A. RNA Isolation from Tissues

1. Add appropriate tissues in a mortar placed in liquid nitrogen and carefully grind.
   While using frozen tissues, you must make sure that all frozen tissues should be frozen forms, during grinding, be cautious that the frozen tissues do not dissolve before adding lysis buffer. Slight melting may result in degradation of RNA.
2. Add appropriate lysis buffer and briefly vortex.

Weight of tissue	10-100 mg	100-300 mg	300-600 mg	600-1000 mg
Tube size	1.5 ml	15 ml	50 ml	50 ml
Lysis buffer	1.0 ml	3.0 ml	6.0 ml	10.0 ml
Chloroform	0.2 ml	0.6 ml	1.2 ml	2.0 ml
Phenol:Chloroform	0.6 ml	1.8 ml	3.6 ml	6.0 ml
Isopropanol	0.6 ml	1.8 ml	3.6 ml	6.0 ml
80% EtOH	0.6 ml	1.8 ml	3.6 ml	Ml

   In the case of Genomic DNA-rich tissue(ex. Lung, pancreas, kidney, placenta), add sufficient lysis buffer.
3. Add appropriate chloroform, and vigorously vortex for 1 min.
4. Proceed the reaction under ice condition for 5 minutes.
5. Centrifuge for 10 minutes at 12,000 rpm, 4 °C.
6. Transfer the supernatant.
7. Add equal volume of Phenol:Chloroform(5:1) and vortex.
8. Centrifuge for 5 minutes, at 14,000 rpm, 4 °C.
9. Transfer the supernatant.
   In the case of RNase-rich tissues(ex.. Pancreas, liver, spleen…), repeat treatment step of phenol:chloroform several times more.
10. Add equal volume of isopropanol and slowly mix.
11. Store at - 20 °C for 10 minutes.
12. Centrifuge at 12,000 rpm, 4 °C for 10 minutes.
13. Discard the supernatant.
14. Add equal volume of 80% EtOH and then tapping.
15. Centrifuge at 12,000 rpm, 4 °C for 5 minutes.
16. Discard the supernatant, then dry the pellet.
    Slightly dry RNA pellet in a clean bench, if dry it too much, it is not easy to dissolve it during suspension.
17. Add appropriate RNA Hydration Solution and DEPC-DW, completely suspend RNA pellet(1 μg/μl).
18. Until treatment of Dnase, store RNA sample at - 70 °C.
    It is recommended to promptly measure the yield and purity of it after extraction of RNA, then proceed the

treatment of DNase, if it will be treated another time for some reasons, suspend RNA using EtOH and store it at -70°C.

B. RNA Isolation from Cultured Cells

1. Centrifuge the cultured cells at 3,000 rpm, 4 °C for 5 minutes.
   While using frozen cells, it is important to maintain the form of frozen cells until adding lysis buffer. Slight melting may result in degradation of RNA.
2. Discard the supernatant, resuspend cells using 1X PBS buffer.
3. Centrifuge cells at 3,000 rpm, 4 °C for 5 minutes.
4. Add appropriate lysis buffer, and vigorously vortex.

Cell number	10^6–10^7	1–3x10^7	3–6x10^7	6–10x10^7
Tube size	1.5 ml	15 ml	50 ml	50 ml
Lysis buffer	1.0 ml	3.0 ml	6.0 ml	10.0 ml
Chloroform	0.2 ml	0.6 ml	1.2 ml	2.0 ml
Phenol:Chlo-roform	0.6 ml	1.8 ml	3.6 ml	6.0 ml
Isopropanol	0.6 ml	1.8 ml	3.6 ml	6.0 ml
80% EtOH	0.6 ml	1.8 ml	3.6 ml	

In order to proceed completed lysis, it is very important to suspend cell pellet completely.

5. Add appropriate chloroform and vigorously vortex for 1min.
6. Proceed reaction at ice condition for 5 minutes.
7. Centrifuge at 12,000 rpm for 10 minutes.
8. Transfer the supernatant.
9. Add equal volume of Phenol:Chloroform(5:1) and vortex.
10. Centrifuge at 14,000 rpm for 5 minutes.
11. Transfer the supernatant.
    In the case of RNase-rich cells (ex. Leukocytes), repeat the treatment step of phenol:chloroform several times more.
12. Add equal volume of isopropanol and slowly mix.
13. Store at −20 °C for 10 minutes.
14. Centrifuge at 12,000 rpm for 10 minutes.
15. Discard the supernatant.
16. Add equal volume of 80% EtOH and then vortex.
17. Centrifuge at 12,000 rpm for 5 minutes.
18. Discard the supernatant, then dry the pellet.
    Slightly dry the RNA pellet in a clean bench, if dry too much, it is not easy to dissolve them during suspension step.
    Add quantitative RNA Hydration Solution and DEPC-DW, then completely suspend the RNA pellet(1 μg/μl).
19. Until the treatment of Dnase, store the RNA sample at − 70 °C.
    It is recommended to promptly measure the yield and purity after extraction of RNA, and then proceed the treatment of RNA, if it will be treated another time for some reasons, suspend RNA using EtOH and store it at -70°C.