Viral RNA PrepMate ™

I. Description

Viral RNA PrepMate™ contains all of the required reagents for easy isolation of total RNA by the guanidinium salt-lysis method. Possible sources include tissue, cultured cells, leukocytes, and serum. The extracted RNA can be used for molecular cloning, RT-PCR, Northern analysis, dot blot hybridization, poly-A (+) selection, RNase protection assays, and so on.

II. Kit Components

Viral RNA PrepMate™(Cat.No.:K-3060)

Lysis Buffer	100 ml
RNA Hydration Solution	10 ml

III. Protocols

A. Extraction of total RNA from tissue

1. Break tissue into smaller units.
2. Add 1ml lysis buffer to 50-100mg tissue
3. Homogenize the sample with a glass-Teflon homogenizer and place at 4℃ for 10 min.
4. Add 750 μl chloroform to the sample. Mix gently and place at 4℃ for 15 min to precipitate proteins.
5. Centrifuge at 13,000 rpm for 20 min at 4℃.
6. Transfer the supernatant and add to it an equal volume of isopropanol. Store at -20℃ for one hour.
   - ■ If the sample is placed at a low temperature (below -20℃) or 2 volumes of ethanol are added, too much salt will be precipitated.
7. Centrifuge at 13,000 rpm for 20 min at 4℃.
8. Wash pellet twice with 70% DEPC-treated ethanol.
9. Remove ethanol completely with cautious aspiration; spinning down may be necessary to force droplets to the tube bottom.
10. Remove remaining traces of ethanol by incubating open tube on a heating block for 5 min at 56℃.
11. Resuspend the pellet in RNA hydration buffer (DEPC treated DW).
12. Add 6 units of DNase I and incubate for 2 hr at 37℃. Inactivate DNase I by heating at 94℃ for 10 min.
13. Quantify the yield of total RNA with a spectrophotometer. Store at -70℃.
    - ■ Use RNA Hydration Buffer as a blank.
    - ■ When OD_{260} is 1.0, the concentration of total RNA is 40ug/ml.

B. Extraction of total RNA from leukocytes or cultured cells

1. Add 1ml lysis buffer to the suspended cells and vortex it gently in an inverted position for 10 seconds.
2. Incubate for 10 min at 4℃.
3. Follow the step III-A4 to III-A13.

C. Extraction of total RNA from serum

1. Add 800ul lysis buffer to 200ul serum.
2. Mix carefully and place at 4℃ for 10 min.
3. Follow the step III-A4 to III-A13.

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