Blood RNA PrepMate

Description:

This kit is designed for rapid isolation of high-purity total RNA from tissue or cells based on the guanidinium salt-lysis method. The extracted total RNA can be used for Northern analysis, dot blot hybridization, poly-A (+) selection, RNase protection assays and molecular cloning, or RT-PCR etc..

System Components

Blood Buffer	100ml
Suspension buffer	50ml
Lysis buffer	100ml
RNA Hydration Solution	10ml

Protocol

Extraction of total RNA from human blood/tissue

1. Add 300ul of whole blood and 900ul of blood buffer to a 1.5ml eppendorf tube, then suspend the mixture by gentle complete vortexing.
2. Incubate the tube at 4℃ for 20 min (During this time, mix the homogenized sample by gentle inversion), then centrifuge the tube at 5,000 rpm for 15 min at room temperature.
3. Discard the supernatant.
   - The precipitant on the bottom is white blood cell, and the surface of the cells appears light red. Add 500ul of blood buffer and wash off the red color inside of the tube. Do not vortex the tube vigorously because treatment of blood buffer may result in a little lysis of the white blood cells, vigorous vortexing can also lyse the white blood cells, ultimately reducing the yield of recovery due to RNA hydrolysis.
4. Centrifuge for 15 minutes at 5,000 rpm, room temperature.
5. Pour off the supernatant, and spin-down again then completely discard the supernatant using tip.
6. Add 200ul of suspension buffer to the withdrawal precipitant, and completely resuspend the precipitant.
   - Add 200ul of suspension buffer for per gram of tissue and smash the tissue by using grinder. Do not votex, mix only by tapping or inversion. If the temperature of suspension buffer decreases, some precipitants may appear. It is important to store suspension buffer at 25℃. If some precipitants appeared, the suspension buffer should be re-dissolved by heating.
7. After complete resuspension of the precipitants, incubate the sample for 20 min at 4℃.
   - Sufficient incubation time allows complete solubilization of protein and increases the effectiveness of the lysis buffer treatment.
8. Add 200ul of lysis buffer to the sample and mix by gentle inversion, then incubate for 15 min at 4℃.
9. Add 150ul of chloroform and mix by gentle inversion, then incubate for 15 min at 4℃.
10. Centrifuge the tube at 13,000rpm, 4℃ for 10 min.
    - If upper layer is separated completely, proteins should appear in the middle layer. In case of complete treatment of suspension buffer, white debris in the middle layer may not be observed, however, in case of short treatment of suspension buffer, too much white debris may be appeared, it is difficult to separate pure RNA due to disturbing the debris layer during transferring the supernatant.
11. Transfer the supernatant and add an equal volume of 2-propanol with sample and place it at -20℃for 1 hrs.
12. Centrifuge the tube at 13,000rpm, 4℃ for 10 min.
13. Wash it twice using 70% ethanol (DEPC treated)
14. After final washing step, carefully pour off supernatant, and spin down it, then remove the reminder of solution in the tube by using tip.
15. Remove residual ethanol by heating the tube in a heating block at 56℃ for 5 min.
16. Dissolve in RNA hydration buffer.
    During dissolving in the RNA hydration buffer, if the sample is dissolved in a small amount of the buffer(Certainly, DNA contamination happened), RNA can not be dissolved even after several days, and still remains inside the tube. It is important to dissolve the RNA using

appropriate amount of the buffer, and add 60uL of the buffer to dissolve RNA inside wall of the tube by tapping. If too much starting blood is used, add appropriate RNA hydration buffer, RNA remained on inside wall may be dissolved overnight. Do not vortex during this step.

17. Add DNase I and incubate at 37℃ for 2 hr, then inactivate DNase I by heating at 94℃ for 10 minutes.
    It is recommended that the addition amount of DNase I should be 2 units per 100uL of blood, and incubation time should be 1-2 hrs, however, if the incubation time is below 1 hr, DNA can not be eliminated completely, if the time is over 3 hrs, the stability of RNA may be reduced. The method of phenol/chloroform extraction is better than the heating method for inactivation of DNase, however, sequential alcohol precipitation after the method of phenol/choroform extraction may result in RNA loss significantly. In case of heating method for inactivation of DNase, the total activity of enzyme cannot be eliminated because of usage of too many enzymes, using minimum amount of enzyme is recommended.

18. Quantify the yield of total RNA using a spectrophotometer and store in a Deep-Freezer at -70℃.
    - RNA hydration buffer is used as Blank, dilute the RNA in RNA hydration buffer and then quantify it, when OD_{260} is 1.0, the concentration of total RNA is 40ug/ml.

Extraction of total RNA from leukocytes/cultured cells and serum

1. Add 1ml of lysis buffer to 5-10X10^6 pelleted cells.
2. Mix by gentle inverting 3-4 times and incubate at 4℃ for 10 minutes.
3. Add 750ul chloroform and mix gently and well. Incubate 4℃ for 5-10 minutes, and centrifuge at 10,000 X g, 4℃ for 10 min, then carefully transfer the supernatant into a new tube.
4. Add equal volume of isopropanol to supernatant and incubate at -20℃ for 1hr and then centrifuge at 10,000 X g, 4℃ for 10 min.
5. Rinse the pellet with 1ml 70% ethanol (DEPC-treated) 2 times, and dry the pellet with a heating block or speed vac for 2-3 min, 56℃.

   And suspend the pellet in an appropriate volume of RNA hydration solution.
6. Make a dilution solution and quantify the yield of total RNA using a spectrophotometer.