Genorise Plsma/Serum Virus RNA Extraction 400 Kit

Genorise Plasma/Serum Virus RNA Extraction Kit is to isolate virus RNA from fresh plasma or serum and for 400 applications. This kit can significantly improve quality and quantity of RNA with a RIN > 8 and is much cheaper than the similar products ($0.4/application). A much higher yield can be expected from this kit in comparison with other methods.

Materials provided:
225 ml of Genorise Reagent A
30 ml of Genorise Reagent C
100 ml of RNA water
5 ml RNA sample loading buffer

Materials required but not provided
Water-saturated phenol
Chloroform-isoamyl alcohol (49:1)
0.2% Diethyl pyrocarbonate (DEPC) water
2-mercaptoethanol
Isopropanol (2-propanol)
Ethanol

Protocol

1. Add 0.5 ml of Genorise Reagent B to 0.5 ml plasma or serum in a 1.5 ml microcentrifuge tube, briefly vortex, incubate 5 min at room temperature.
2. Centrifuge 5 min at 5000 x g to remove 0.2 ml cell debris and transfer to new 15 ml tube.
3. Sequentially add 0.1 ml Reagent C, vortex 15 sec; 1 ml water-saturated phenol, vortex 15 sec and 0.2 ml chloroform-isoamyl alcohol, vortex 15 sec.
4. Incubate on ice for 15 min and aliquote to two 1.7 ml microcentrifuge tubes, and centrifuge at no more than 12,000 x g at 4°C for 15 min.
5. Transfer the upper aqueous phase (0.5 ml) containing RNA to new tube containing 0.5 ml of isopropanol, close the cap and invert 50 times, and incubate at room temperature for 10 min to precipitate RNA.
6. Centrifuge at no more than 12,000 x g at 4°C for 10 min.
7. Wash the RNA pellet with 1ml of 75% ethanol at no more than 7500 x g at 4°C for 5 min and repeat the wash once.
8. Discard the supernatant, remove the residue liquid by a pipette and air dry for 5 min.
9. Add 30 µl of DEPC-treated water to completely dissolve the RNA pellet by a pipette or brief vortex, and combine the aliquotes.
10. Read at A_{260} and A_{280} and run RNA electrophoresis to estimate RNA quality and quantity. Store the RNA samples at -80°C.

Reagent preparations

Genorise Reagent B: Add 0.36 ml of 2-mercaptoethanol to 50 ml of Genorise Reagent A and use it within a month.

Water-saturated phenol: Dissolve 30 g phenol crystal in 20 ml of 0.2% DEPC water at 60°C. Aspirate the upper water phase, store at 4°C and use it within a month.

Prepare 0.2% DEPC water, stir in the hood overnight, and autoclave at 120°C for 30 min.
Prepare 50 ml of chloroform-isoamyl alcohol (49:1) in glass bottle.
Prepare 75% ethanol in 0.2% DEPC-treated water

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Additional Protocols

Quantitate RNA
Quantitate RNA by diluting 5 µl in 0.195 ml of 1 mM Na_2HPO4 solution (pH >7.5) and reading the A_{260} and A_{280}. The ratio of the A_{260} and A_{280} should be >1.8. Do not used distilled water because it has pH <6.

RNA Electrophoresis to evaluate the RNA quality

Reagent preparation
10 x MOP Running Buffer: 0.4 M MOPs, 0.1 M Sodium Acetate, 0.01 M EDTA
1 x Formaldehyde Gel Running Buffer: 1 x MOP Running Buffer, 0.61% formaldehyde

1% agarose/formaldehyde gel
1g agarose in 87 ml H2O, heat 1 min in microwave to completely melt the agarose, add 10 ml 10 x MOP running buffer and 3 ml of 12.3 M formaldehyde, mix, cool down and pour to the gel tray.

Add sufficient 1 x Formaldehyde gel running buffer to cover the gel

Prepare sample and run gel
1. Mix 5 µl RNA with 15 µl RNA sample buffer
2. Denature at 65ºC for 10 min
3. Chill on ice at least 5 min
4. Run the gel at 10 V/cm until the bromphenol blue dye migrate one-half length of the gel.

Visualization of RNA under UV light: Photograph the RNA gel

Technical notes
1. The white cell pellet should be free of RBC (no or little red remain) prior to addition of Reagent B.
2. Removal of RBC should be a quick process to avoid RNA degradation.
3. Water-saturated phenol should be used within a month otherwise RNA isolation may fail.
4. Air drying of RNA pellet should not exceed 5 min.
5. RNA yield is usually 4 µg/ml to 8 µg/ml blood depending on species. Normal $A_{260/280}$ is approximately 2.0. If less or more blood is used, the reagents should be reduced or increased accordingly.