Genorise Blood RNA Isolation (300)

Genorise Blood RNA Isolation kit is to isolate RNA from fresh blood and for 300 applications. This kit can significantly improve quality and quantity of RNA with a RIN > 8 and is much better and cheaper than the similar products such as Trizol Reagent. A much higher yield can be expected from this kit in comparison with other filter based methods.

Materials provided:
400 ml of 10x Red Blood Cell (RBC) Lysis Buffer
230 ml of Genorise Reagent A
3 ml Reagent C
3 ml Reagent D
100 ml of RNA water
5 ml RNA sample loading buffer

Materials required but not provided
Water-saturated phenol
Chloroform
Diethyl pyrocarbonate (DEPC)
2-mercaptoethanol
Isopropanol (2-propanol)
Ethanol

Protocol

1. Dilute 10 x RBC Lysis Buffer to 1 x by RNA water.
2. Collect 5 ml of fresh blood in 10 ml glass tube containing anti-coagulant and spin 10 min at 1000 x g at 4°C.
3. Discard plasma and decant blood to a 50 ml conical tube.
4. Add 5 ml of 1 x RBC Lysis Buffer (RNA) to the original glass tube and vortex briefly and decant to the 15 ml tube. Add additional 5 ml 1 x RBC Lysis Buffer to the blood, vortex 20 sec and incubate 7-10 min at room temperature to completely disrupt the red blood cells (the blood lysate should become clear).
5. Centrifuge at 3500 x g for 5 min at 4°C and remove the blood lysate.
6. Suspend the white cell pellet in 1 ml of 1 x RBC Lysis Buffer and transfer to a 1.5 ml tube.
7. Centrifuge at 5000 x g for 1 min at 4°C to completely remove the RBC.
8. Add 0.7 ml of Genorise Reagent B and resuspend immediately to completely lyse the cells by a pipette or disrupt the cell pellet by sonication on ice at low speed for **no more than 5 seconds**, incubate 15 min on ice.
9. Sequentially add 0.7 ml water-saturated phenol, vortex 15 sec and 0.3 ml chloroform, vortex 15 sec.
10. Incubate on ice for 15 min and centrifuge at no more than 12,000 x g at 4°C for 15 min.
11. Transfer the upper aqueous phase (0.7 ml) containing RNA to new tube containing 0.7 ml of isopropanol, close the cap and invert 50 times, and incubate at room temperature for 10 min to precipitate RNA.
12. Centrifuge at no more than 12,000 x g at 4°C for 10 min.
13. Wash the RNA pellet with 1.5 ml of 75% ethanol at no more than 7500 x g at 4°C for 5 min and repeat the wash once.
14. Discard the supernatant, remove the residue liquid by a pipette and air dry for 5 min.
15. Add 50 µl of DEPC-treated water to completely dissolve the RNA pellet by a pipette or brief vortex.
16. Read at A_{260} and A_{280} and run RNA electrophoresis to estimate RNA quality and quantity. Store the RNA samples at -80°C.

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Reagent preparations

Genorise Reagent B: Add 0.35 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 RNA water at 60°C. Aspirate the most upper water phase and add 0.3 ml Reagent C and 0.3 ml reagent D, store at 4°C and use it within a month.

RNA Water: Prepare 0.02% DEPC water, stir in the hood overnight, and autoclave at 120°C for 30 min.
Prepare 75% ethanol in RNA water

Alternative protocol for Isolation of White Blood Cells by White Buffy Coat

1. Collect 5 ml blood in a 10 ml glass tube containing anti-coagulant such as heparin, centrifuge 10 min at 1000 x g.
2. Pick up the white Buffy coat layer (no more than 1 ml) by a Pasteur pipette and place into a 15 ml conical tube containing 4 ml red blood cell lysis solution, briefly vortex to suspend the cells.
3. Incubate 7-10 min at room temperature until the cell lysate becomes clear (do not incubate more than 10 min).
4. Spin 3 min at 1000 x g to pellet the white blood cells and discard the supernatant.
5. Remove the liquid residue and add 1 ml red blood cell lysis solution to suspend the white cells.
6. Transfer the cells to a 1.5 microcentrifuge tube and spin 1 min at 3000 x g.
7. Completely remove the supernatant and suspend the cells with 1ml PBS.
8. Spin 1 min at 3000 x g and completely remove the PBS.

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 (or purchase from GENORSE SCIENTIFIC INC, Cat. 102014)
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

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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. Do not sonicate to disrupt the cells more than 5 seconds, otherwise RNA may encounter degradation.
5. Air drying of RNA pellet should not exceed 5 min.
6. RNA yield is usually 3 μg 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.