



GSI Blood RNA Isolation (500)

GSI Blood RNA Isolation (500) kit is to isolate RNA from fresh blood and for 500 applications. This kit can significantly improve quality and quantity of RNA with a RIN > 8 and is much better (28S > 18S) and cheaper than the similar products such as Trizol Reagent (28S < 18S). A much higher yield can be expected from this kit in comparison with other filter based methods.

Materials provided:

100 ml of 50x Red Blood Cell (RBC) Lysis Buffer
500 ml of GSI RNA Reagent
200 ml of RNA water
5 ml RNA sample loading buffer

Materials required but not provided

Chloroform
Diethyl pyrocarbonate (DEPC)
Isopropanol (2-propanol)
Ethanol

Protocol

1. Dilute 50 x Red Blood Cell Lysis Solution 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 15 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 50 ml tube. Add additional 5 ml of 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 1 ml of GSI RNA Reagent and resuspend immediately to completely lyse the cells by a pipette or to disrupt the cells by sonication on ice for **no more than 10 seconds** at low power, incubate 15 min on ice.
9. Add 0.2 ml chloroform, vortex 15 sec. and incubate on ice for 15 min.
10. Centrifuge at no more than 12,000 x g at 4°C for 15 min.
11. 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.
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 RNA water to completely dissolve the RNA pellet by a pipette or brief vortex.
16. Read at A₂₆₀ and A₂₈₀ and run RNA electrophoresis to estimate RNA quality and quantity. Store the RNA samples at -80°C.



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Reagent preparations

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 Na₂HPO₄ solution (pH >7.5) and reading the A₂₆₀ and A₂₈₀. The ratio of the A₂₆₀ and A₂₈₀ should be >1.8. Do not use 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 65°C 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 GSI RNA Reagent.
2. Removal of RBC should be a quick process to avoid RNA degradation.
3. Do not sonicate to disrupt the cells more than 10 seconds, otherwise RNA may encounter degradation.
4. Air drying of RNA pellet should not exceed 5 min.
5. RNA yield is usually 3 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.