

RNA and miRNA Isolation from Human Peripheral Blood

Note: This protocol assumes the investigator is beginning this with one full Yellow-Top (type A) BD Vacutainer tube of human blood (equals roughly 8 ml) to yield approximately 30 ug of RNA.

Additional Note: RNA is very easily degraded by ever-present RNAses. Therefore, all of the tubes and solutions in this protocol must be RNase-free (autoclaving does NOT inactivate RNAses). One cannot overemphasize the need for a clean work environment when working with RNA.

- 1) Transfer contents of tube into a 50 ml polypropylene conical centrifuge tube.
- 2) Bring volume to 45 ml with RBC Lysis Buffer (recipe follows protocol).
- 3) Let stand at room temperature for 10 minutes.
- 4) Pellet cells at 600 x g (approx 1,400 rpm) for 10 minutes in a room temp centrifuge (program #3).
- 5) Carefully decant supernatant.
- 6) Gently resuspend the pellet in 1 ml of RBC Lysis Buffer and transfer to a 1.5 ml microcentrifuge tube. – Let stand for 5 minutes.
- 7) Pellet cells for 2 minutes by centrifuging in a microfuge at room temperature at 3000 rpm.
- 8) Carefully aspirate the supernatant.
- 9) Resuspend the pellet in 1 ml of sterile DPBS.
- 10) Pellet cells as in step 7.
- 11) Carefully aspirate the supernatant.
- 12) Add 1200 µl of TRIzol solution to each tube and resuspend the cells. Note: for a full 8 ml blood tube, the 1200 ul TRIzol solution can be split into 2, 600 µl aliquots and frozen at -80 C until further processing.
- 13) Add 0.2 ml of Chloroform (CHCl₃) and vortex each tube for 15 seconds, ONE AT A TIME.
- 14) Centrifuge the samples at 13,000 rpm for 10 minutes at 4°C.
- 15) Remove the upper phase and transfer to a clean microcentrifuge tube. Be careful not to remove any of the white interface when collecting the upper phase of the extraction
- 16) For the future collection of micro RNA (miRNA), carefully remove ~20% of the volume of the upper phase from step 16 and place into another clean, labeled, 1.5ml microfuge tube. Store this aliquot at -80 C until further processing.
- 17) To the remaining upper phase from step 16, add an equal volume of cold isopropanol and invert to mix.
- 18) The samples can be placed in a -20°C freezer to precipitate.
- 19) Samples are centrifuged at 13,000 rpm for 10 minutes at 4°C. Note: you may be able to see a small white pellet of RNA at the bottom of the tube after this step.
- 20) Carefully decant the supernatant, and rinse the pellet with 0.5 ml of ice-cold 75% ethanol. The 75% EtOH should be prepared RNase-free and stored at -20 C.
- 21) Centrifuge the samples at 13,000 rpm for 10 minutes at 4°C.
- 22) Decant the supernatant.
- 23) Using a pipettor, carefully remove all of the remaining liquid in the bottom of the tube.

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- 24) Allow the pellet to dry for 5 to 10 minutes to remove any remaining ethanol.
- 25) Dissolve the RNA pellet by adding 20 μ l of RNase-free H₂O to each sample.
- 26) RNA should be quantitated within 2 hours of elution. It can be kept at 4 C until that time; it can also be held temporarily at -20 until permanent storage at -80. Repeated freeze-thaws are to be avoided, so RNA should be aliquoted for transfer as soon as possible after quantitation.

10x RBC Lysis Buffer

89.9 g NH₄Cl
10.0 g KHCO₃
2.0 ml 0.5 M EDTA

Dissolve the above in approximately 800 ml ddH₂O and adjust pH to 7.3. QS to 1 liter and mix thoroughly. This solution is stable for 6 months at 2 – 8° C in a tightly closed bottle.

1x RBC Lysis Buffer

Simply dilute the 10x stock solution 1:10 with ddH₂O. Stable for 1 week at room temperature.

TRIzol Reagent

Invitrogen Life Technologies: Cat No. 15596018

OR

RNA STAT-60 Reagent

Tel-Test: Cat No. CS-111

Other Reagents Needed

Phosphate Buffered Saline (PBS)

Isopropanol (2-propanol)

Ethanol

RNase-free water

RNase-Away (a cleaning solution that neutralizes RNAses on bench tops, pipettors, centrifuges, and other equipment.