# 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 ($CHCl_3$) 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 (~40 uL) 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 (~ 200 uL) invert to mix.
18) The samples are placed in a -20°C freezer to precipitate for 30 minutes.
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.

24) Allow the pellet to dry for 5 to 10 minutes to remove any remaining ethanol.
25) Dissolve the RNA pellet by adding 20 µl of RNAse-free $H_2O$ 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 $NH_4Cl$
10.0 g $KHCO_3$
2.0 ml 0.5 M EDTA

Dissolve the above in approximately 800 ml $ddH_2O$ 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 $ddH_2O$. 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 destroys RNAses).
Chloroform (CHCl3)