

**Protocol Name: RNA CPT v1**

Notes:

- draw 8ml of blood into the CPT Tube, invert the tube 10 times immediately after draw, do not shake, keep at room temperature, and centrifuge within 2 hours
  - bring Trizol, isopropanol to room temperature
  - cool eppendorf centrifuge
  - set BioOven to 55°C
  - initial 10 steps below must be performed prior to Total RNA Isolation
1. Centrifuge within 2 hours from the time of blood draw in a centrifuge with a swing-out bucket rotor for 20 minutes, room temperature at 1700g.
  2. Using 3ml transfer pipet, aliquot approximately 3ml of clear plasma from the uppermost layer to 4ml tube, avoid disturbing the whitish cell layer, and freeze at -20°C.
  3. Recap the tube with the stopper and invert 10 times. Pour off the cell/plasma mixture into a 15ml tube.
  4. Add PBS to bring the volume to 15ml, cap tube, and mix cells by inverting tube 5 times.
  5. Centrifuge for 15 minutes, room temperature at 300g. Aspirate as much supernatant as possible without disturbing the cell pellet, leaving a few microliters of supernatant with the cell pellet.
  6. Resuspend the pellet by gently vortexing or tapping tube with finger.
  7. Add PBS to bring volume to 10ml, cap tube, and mix cells by inverting 5 times.
  8. Centrifuge for 10 minutes, room temperature at 300g. Aspirate as much supernatant as possible without disturbing the cell pellet, leaving a few microliters of supernatant with the cell pellet.
  9. Resuspend the pellet by gently vortexing or tapping tube with finger.
  10. Add 1ml of RNAlater solution to the cell pellet, resuspend, and then transfer into 2ml tube for storage at -20°C until Total RNA Isolation is to be performed.

*Total RNA Isolation:*

1. Centrifuge PBMC/RNAlater mixture at 14,000g, room temperature, for 2 minutes to pellet cells.
2. Remove supernatant using pipet.
3. In fume hood, add 1ml Trizol.
4. Using a 23G needle attached to a 5ml syringe, homogenize the cells by carefully aspirating and dispensing the Trizol through the needle, check visually to ensure complete homogenization. Wear eye protection during this step, and do not recap the needle.
5. In fume hood, add 200ul Chloroform, cap securely, vortex lightly for 15-20 seconds.
6. Spin at 12,000g, 4°C, for 10 minutes.
7. Carefully remove upper aqueous layer down to the interphase using a P200 pipet tip and transfer into a new 1.5ml tube (~500ul volume). Save the tubes containing

- the Trizol/Chloroform mixture for subsequent DNA and protein extraction (freeze the sample if the isolation of DNA and protein will be done another day).
8. Add 500ul of room temperature isopropanol and mix by inversion (add more isopropanol to tubes that have total volumes less than 1ml).
  9. Incubate at room temperature for 10 minutes.
  10. Spin at 12,000g, 4°C, for 10 minutes.
  11. Carefully decant the supernatant by pouring off.
  12. Add 500ul 70% ETOH, do not resuspend pellet.
  13. Spin at 7,000g, 4°C, for 5 minutes.
  14. Carefully decant the supernatant by pouring off and turn tubes upside down on Kim-wipes. Using a P10 pipet tip, aspirate any remaining ETOH and then immediately add 100ul DEPC water. Do not allow the RNA pellet to dry completely, do not speed-vac, do not resuspend the pellet.
  15. Incubate the tubes at 55°C in BioOven for 10 minutes.
  16. Pellet usually dissolves by itself, tap the tube a couple times and then quick spin. Store sample at 4°C or -20°C until ready to perform the RNeasy clean up.
  17. In fume hood, make up enough RLT buffer with B-mercaptoethanol for prep (10ul BME : 1ml RLT buffer).
  18. Add 350ul RLT to each tube containing sample (total RNA in 100ul water).
  19. Add 250ul cold 100% ETOH, mix.
  20. Add to RNeasy column.
  21. Spin 10,000g, room temperature, for 1 minute.
  22. Reapply flow through to column by pouring off.
  23. Spin 10,000g, room temperature, for 1 minute.
  24. Place column in a new 2ml collection tube.
  25. Add 500ul RPE buffer.
  26. Spin 10,000g, room temperature, for 1 minute.
  27. Discard flow through.
  28. Add 500ul RPE buffer.
  29. Spin 10,000g, room temperature, for 1.5 minutes.
  30. Discard flow through.
  31. Spin 10,000g, room temperature, for 2 minutes.
  32. Heat an aliquot of water to 70°C.
  33. Place column in a new 1.5ml tube.
  34. Add 50ul of 70°C water to the membrane, incubate at room temperature for 1 minute.
  35. Spin 14,000rpms, room temperature, for 2 minutes.
  36. Quantitate on NanoDrop.