Parkinson's Progression Markers Initiative

# PPMI PBMC Highlights

- Samples are collected in a 10 mL Sodium Heparin (green top) tube at the visits below:
  - BL, V02, V05, V06, V07, V09, V11, V13, V14-V20
- Sites ship samples out on the day of collection for next-day delivery at the repository for immediate processing
  - Samples are shipped on refrigerant packs in order to maintain a constant temperature
  - Samples may be up to approximately 24 hours old at the time of receipt
- Approximately 2 million cells are frozen per 1 mL vial (see detailed procedure)
  PBMCs are shipped in 6.5% DMSO
- On average, PPMI generates 3.7 (approx. 4) PBMC aliquots from one, 10 mL NaHep tube

# PPMI PBMC Isolation

1. Obtain whole blood collected in a 10 mL NaHep tube
   a. Set up consumables
      i. 1 x ACCUSPIN™ cell separation tube
      ii. 1 x 50 mL conical tube
      iii. 1 x cell counting sheet
   b. Label all consumables with the corresponding barcode
2. Perform all work in a Biological Safety Cabinet.
3. Add 15 mL of Histopaque® to the ACCUSPIN™ tube.
4. Centrifuge the ACCUSPIN™ tube to bring the Histopaque® below the frit. Spin the tube at 1044 ± 20 xg for 30 seconds – 1 minute.
5. Obtain all the whole blood from the NaHep tube and transfer it to the ACCUSPIN™ cell separation tube.
6. Rinse out the NaHep tube with a 1:1 volume of RPMI-1640 plus HEPES. Transfer the RPMI and blood mixture to the ACCUSPIN™ tube.
7. Centrifuge the ACCUSPIN™ tube at 1044 ± 20 xg at room temperature for 10 minutes with the brake OFF.
8. After centrifugation, discard the top plasma layer. Collect the cell layer and transfer it to the labeled 50 mL conical tube.
9. Add the RPMI wash media to bring the total volume up to 10 – 12 mL.
10. Centrifuge at 600 ± 20 xg at room temperature for 10 minutes with the break on.
11. Discard the supernatant and re-suspend the cell pellet in 10 – 12 mL of RPMI wash media.
12. Centrifuge at 600 ± 20 xg at room temperature for 10 minutes with the break on.
13. Discard the supernatant.
14. Re-suspend the cells in 2 mL of RPMI wash media for cell counting.
15. Record the live cell count and the cell viability on the cell counting sheet. Determine how many vials to freeze.

a. Freeze approximately 2 million cells per vial.

16. Bring the volume of the cells from step 14 up to 10 – 12 mL with the RPMI wash media.
17. Centrifuge at 600 ± 20 xg at room temperature for 10 minutes with the break on.
    a. During the centrifugation, ensure enough freeze media has been thawed for cryopreservation. Freeze media contains 6.25% DMSO, 31.25% FBS, 62.5% RPMI 1640 plus HEPES.
    b. Prepare Mr. Frosty for control rate cryopreservation.
18. Discard the supernatant. Re-suspend cell pellet in appropriate amount of freeze media from the cell counting sheet.
19. Aliquot cells into labeled 1.2 mL cryovials, 1 mL per tube.
20. Place cryovials in the Mr. Frosty.
21. Freeze the cells in -80°C Freezer overnight.
22. Store cells in LN2 the next day.

References: