Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Induced pluripotent stem cells (iPSCs) can be generated from freshly collected or previously frozen peripheral blood (PB) or bone marrow (BM) mononuclear cells (MNCs).

**Materials and reagents:**
- Chlorhexidine chloraprep applicators (Fisher Cat# 14-910-43)
- Sterile Alcohol Prep Pads (Dynarex 1103)
- BD vacutainer CPT cell preparation tubes with sodium citrate (BD Cat# 362760): http://www.bd.com/vacutainer/pdfs/bd_cpt_VDP40104.pdf
- Dulbecco's phosphate buffered saline, D-PBS (Invitrogen Cat# 14190-144)
- Fetal Bovine Serum (FBS) Hyclone Defined (Cat# SH30070.03), 0.22 micron filter before use
- DMSO (Sigma Cat #D2650)
- Cryovials (Corning Cat# 430487)
- 15 mL conical centrifuge tubes (Fisher Cat# 14-959-70C)

1. Clean venipuncture site and top of blood draw tube thoroughly with chlorhexidine prep or 70% isopropyl alcohol pads and allow to dry.

2. Draw 4 mLs of peripheral blood into each of two BD Vacutainer Cell Preparation Tubes (CPTs) with sodium citrate. **Invert the tubes 8 to 10 times** immediately and keep upright at room temperature (RT)

3. Centrifuge at 1,800 x g for 30 min at RT. Ideally, this step should be done within 2 hrs of collection.

4. Using a 1 mL pipette tip, collect the mononuclear cells (MCs) by pipetting the buffy coat (cell layer between gel barrier and plasma) into a sterile 15 mL conical centrifuge tube.

5. Bring total volume to 10 mLs with sterile DPBS and pipette gently or invert several times to mix.

6. Centrifuge at 300 x g for 15 min at RT and aspirate supernatant

7. Resuspend cell pellet in 10 mL of sterile DPBS and perform cell count

8. Resuspend cell pellet in fetal bovine serum (FBS) containing 10% DMSO to freeze. Aliquot ~2x10^6 cells/cryovial in 1 mL volume. Place cryovials in a pre-chilled freezing container and freeze at -80°C overnight. Transfer cryovial to liquid nitrogen storage the following day for long term storage. Typical yield is ~1x10^6 cells/mL blood drawn; for 8 mLs blood drawn, should obtain 6-8x10^6 cells for 3-4 frozen cryovials.
