Audubon
BIOSCIENCE

Standard Processing Protocol for Peripheral Blood Mononuclear Cells (PBMCs) Isolation

1. Using an aseptic technique, obtain a sufficient volume (at least 20mL) of venous blood. Temperate the blood samples, Ficoll-PaqueTM density gradient and Dulbecco's Phosphate-Buffered Saline (DPBS) and Fetal Bovine Serum (FBS) to room temperature.

2. Dilute whole blood sample with an equal volume of DPBS containing 2% FBS.

3. In a 50 mL conical centrifuge tube, layer 25-30 mL of diluted whole blood on top of a pre-layered 15mL Ficoll-PaqueTM Plus density gradient.

4. Centrifuge the layered conical tubes at 1200 xg for 10 minutes at 20°C, with a swing-out rotor at maximum acceleration and breaks OFF.

5. Aseptically aspirate the top plasma layer. Carefully aspirate the whitish fraction corresponding to the PBMCs into a fresh 50 mL conical tube.

6. Dilute PBMCs with 2% FBS to bring volume to 50mL and centrifuge at 300xg for 10 minutes at 20°C.

7. Remove supernatant.

8. If needed, perform red blood cell lysis on the PBMCs pellet by resuspending it in 4 mL red cell lysis buffer and incubating for 5 minutes. Add 20 mL of 2% FBS DPBS to stop the reaction.

9. Centrifuge at 300xg for 10 min at RT and brake ON.

10. Discard the supernatant and resuspend the cells in 1 mL of DPBS supplemented with 2 % FBS. Determine the cell count and viability using a hemocytometer and Trypan blue exclusion method.

11. Wash cells with 20 mL of DPBS and centrifugate at 300xg for 10 minutes at 20°C.

12. Remove supernatant and resuspend cells in 1ml Fetal Bovine Serum (FBS) with 10% Dimethyl Sulfoxide (DMSO) to a final concentration of 10million (1x10^7) cells per mL.

13. Place the cryovials at -80°C overnight in a Mr. Frosty contailer for a controlled freeze down (approximately 1°C per minute).

14. The next day, move the cryovials to a vapor-phase liquid nitrogen storage tank for storage until shipment. Ship on dry ice (-80°C).