DISCOVERY
LIFE SCIENCES

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Standard Processing of Peripheral Blood Mononuclear Cells (PBMCs)

Parameters:

- **Storage Temperature -** LN2 storage
- **Final Product Volume -** 1.0mL
- **Final Product Freezing Media -** 90% heat inactivated FBS/10% DMSO
- **Final Product Vial -** 1.0mL Matrix cryovial (Thermo Fisher catalog number 3740)
- **Cell Count & Viability -** Performed using a Nexcelom Cellometer with AOPI staining

Procedure:

1. Dilute whole blood with dPBS + 2% FBS.

2. Layer diluted whole blood onto SepMate™ tubes containing 15mL Ficoll-Paque™ Plus.

3. Spin layered SepMate™ tubes at 1200xg for 10 minutes at 20°C.
   a. Acceleration = maximum
   b. Deceleration = 60% of maximu

4. Aseptically pipette off and discard plasma layer.

5. Pour PBMC layers into fresh 50mL conical tubes.

6. Dilute PBMCs with dPBS + 2% FBS.

7. Spin cells at 300xg for 10 minutes at 20°C.
   a. Acceleration = maximum
   b. Deceleration = maximum

8. Remove supernatant. (If necessary – a red blood cell lysis step may be performed.)

9. Resuspend pellet with dPBS + 2% FBS and count using the Nexcelom Vision cellometer after AOPI staining.

Standard Processing of Peripheral Blood Mononuclear Cells (PBMCs) - Continued

10. Spin cells at 300xg for 10 minutes at 20°C.
    a. Acceleration = maximum
    b. Deceleration = maximum

11. Remove supernatant.

12. Aseptically resuspend in appropriate volume of 90% FBS/10% DMSO to achieve desired cell density per mL.
    **Depending on the starting total cell count of the sample, vials will be aliquoted 5-10 million viable cells per mL pre-freeze. **

13. Aseptically pipette 1.0mL of PBMCs into labeled 1.0mL Matrix cryovials.

14. Place cryovials into an insulated container and place at -80°C overnight for a controlled freeze down.

15. Move cryovials to a liquid nitrogen storage tank for storage until shipment.