



DISCOVERY

L I F E S C I E N C E S

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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 maximum
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.
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.