

# Standard Processing of Bone Marrow Mononuclear Cells (BMMCs)

|                      |   |
|----------------------|---|
| Donor Type           | Normal & Diseased   |
| Collection Method    | Bone Marrow Aspirate  |
| Processing Method    | Density Gradient via SepMate                                |
| Red Blood Cell Lysis | Yes   |
| Counting Method      | AOPI on Nexcelom Cellometer                                 |
| Freezing Media       | 90% HI-FBS/10% DMSO (Pre 2024)<br>CryoStor CS10 (Post 2024) |
| Product Volume       | 1.0mL   |
| Product Vial         | 1.0mL Matrix Cryovial                                       |
| Storage Temperature  | Liquid Nitrogen Vapor Phase                                 |

## BMMC SepMate Procedure

- Dilute bone marrow with dPBS + 2% FBS.
- Layer diluted bone marrow onto SepMate™ tubes containing 15ml Ficoll-Paque™ Plus.
- Spin layered SepMate™ tubes at 1200xg for 10 minutes at 20°C, acceleration at maximum, deceleration at 60% of maximum.
- Pipette off and discard plasma layer.
- Pour BMMC layers into fresh 50ml conical tubes.
- Dilute PBMCs with dPBS + 2% FBS.
- Spin cells at 300xg for 10 minutes at 20°C, acceleration and deceleration at maximum.
- Remove supernatant.
- Resuspend pellet in 1X Red Blood Cell Lysis Solution.
- Incubate for 10 minutes at room temperature.
- Spin cells at 300xg for 10 minutes at 20°C, acceleration and deceleration at maximum.
- Resuspend pellet with dPBS + 2%FBS and count using acridine orange/propidium iodide on a Nexcelom Cellometer.
- Spin cells at 300xg for 10 minutes at 20°C, acceleration and deceleration at maximum.
- Remove supernatant.
- Resuspend in appropriate volume of cryopreservation media 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\*\*
- Aseptically pipette 1.0ml of BMMCs into labeled 1.0ml Matrix cryovials.
- Place cryovials into an insulated container and place at -80°C overnight for a controlled freeze down.
- Move cryovials to a liquid nitrogen storage tank for storage until shipment.