DISCOVERY LIFE SCIENCES™

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) CryoStor CS10 (Post 2024)
Product Volume	1.0mL
Product Vial	1.0mL Matrix Cryovial
Storage Temperature	Liquid Nitrogen Vapor Phase

BMMC SepMate Procedure

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

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