DISCOVERY LIFE SCIENCES

Standard Processing of Peripheral Blood Mononuclear Cells (PBMCs)

Donor Type	Normal & Diseased	Normal & Diseased
Collection Method	Whole Blood	Leukapheresis
Processing Method	Density Graident via SepMate	Density Gradient
Red Blood Cell Lysis	Yes	Yes
Counting Method	AOPI on Nexcelom Cellometer	AOPI on Nexcelom Cellometer
Freezing Media	90% HI-FBS/10% DMSO (Pre 2024) CryoStor CS10 (Post 2024)	CryoStor CS10
Product Volume	1.0mL	1.5ml
Product Vial	1.0mL Matrix Cryovial	2.0ml Corning Tube (Normal) 4.0ml Coming Tube (Diseased)
Storage Temperature	Liquid Nitrogen Vapor Phase	Liquid Nitrogen Vapor Phase

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

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Leukapheresis PBMC Procedure

1. Drain the leukopak into a 250ml conical tube.
2. Spin cells at 500xg for 10 minutes at 20°C, acceleration and deceleration at maximum.
3. Remove supernatant.
4. Resuspend in dPBS + 2% FBS + 1mM EDTA.
5. Layer diluted blood onto 50mL conical tubes containing 15mL LymphoPrep.
6. Spin layered 50mL conical tubes at 800xg for 20 minutes at 20°C, acceleration at 20% of maximum, deceleration at zero.
7. Pipette off and discard top layer.
8. Carefully transfer to white, PBMC interface into new 250mL conical tubes.
9. Add dPBS + 2% FBS + 1mM EDTA to 250mL.
10. Spin cells at 300xg for 10 minutes at 20°C acceleration and deceleration at maximum.
11. Remove supernatant.
12. Repeat steps 9-11 twice for three total washes.
13. Resuspend pellet in Ammonium Chloride Solution.
14. Incubate for 15 minutes on ice.
15. Spin cells at 300xg for 10 minutes at 20°C, acceleration and deceleration at maximum.
16. Resuspend pellet with dPBS + 2% FBS +1mM EDTA and count using acridine orange/ propidium iodide on a Nexcelom Cellometer.
17. Spin cells at 300xg for 10 minutes at 20°C, acceleration and deceleration at maximum.
18. Remove supernatant.
19. Resuspend in appropriate volume of CryoStor CS10 to achieve desired cell density per mL.
    ** Vials are generated at 15 million, 25 million, 50 million, and 100 million cells (normal collections) or 300 million cells (diseased collections). **
20. Aseptically pipette 1.5mL PBMCs into labeled 2.0mL or 4.0mL Corning tubes.
21. Place cryovials into an insulated container and place at -80°C overnight for a controlled freeze down.
22. Move cryovials to a liquid nitrogen storage tank for storage until shipment.

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