



FOLIO CONVERSANT

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Standard Processing of Diseased Bone Marrow Mononuclear Cells (BMMCs)

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 bone marrow with dPBS + 2% FBS.
2. Layer diluted bone marrow into 50mL conical tubes containing Ficoll-Paque™ Plus.
3. Spin layered tubes at 400xg for 20 minutes at 20°C.
 - a. Acceleration = 10% of maximum
 - b. Deceleration = 0
4. Aseptically pipette plasma buffy coat layer into a fresh 50mL conical tube.
5. Dilute BMMCs with dPBS + 2% FBS.
6. Spin cells at 300xg for 10 minutes at 20°C.
 - a. Acceleration = maximum
 - b. Deceleration = maximum
7. Remove supernatant. (If necessary – a red blood cell lysis step may be performed.)
8. Resuspend pellet with dPBS + 2% FBS and count using the Nexcelom Vision cellometer after AOPI staining.
9. Spin cells at 300xg for 10 minutes at 20°C.
 - a. Acceleration = maximum
 - b. Deceleration = maximum

Standard Processing of Diseased Bone Marrow Mononuclear Cells (BMMCs) - Continued

10. Remove supernatant.
11. 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. **
12. Aseptically pipette 1.0mL of BMMCs into labeled 1.0mL Matrix cryovials.
- 13.. Place cryovials into an insulated container and place at -80°C overnight for a controlled freeze down.
14. Move cryovials to a liquid nitrogen storage tank for storage until shipment.