Audubon
BIOSCIENCE

Standard Processing Protocol for Bone Marrow Mononuclear Cells (BMMCs) Isolation

1. Dilute the collected bone marrow aspirate in a 1:1 ratio with PBS (Phosphate-buffered saline).

2. Fill a 15 ml centrifuge tube with 4 ml Ficoll-Paque density gradient.

3. Carefully layer the diluted bone marrow sample on top of the density gradient fraction.

4. Using a swing-out rotor, centrifuge for 20 min at 1200xg at room temperature, with the brake OFF.

5. Aspirate the top supernatant layer. Collect and transfer the whitish BMMC fraction in a new 15 ml centrifuge tube and resuspend it with 5 ml RPMI medium containing 1% FBS.

6. Fill up the 15ml centrifuge with 1% FBS RPMI.

7. Centrifuge at 300xg for 8 min at 4°C.

8. Aspirate the supernatant and wash the cell pellet with 15ml 1% FBS RPMI and centrifugate at 300xg for 5 min at 4°C.

9. Aspirate the supernatant and resuspend the BMMCs pellet in 4 ml of 1% FBS RPMI medium.

10. Count the cells and proceed for downstream analysis or cryopreservation.

11. For cryopreservation, BMMCs are resuspended in 1ml Fetal Bovine Serum (FBS) with 10% Dimethyl Sulfoxide (DMSO) to a final concentration of 50 million (5x10^7) cells per mL.

12. Place the cryovials at -80°C overnight in a Mr. Frosty for a controlled freeze down (approximately 1°C per minute).

13. The next day, move the cryovials to a vapor-phase liquid nitrogen storage tank for storage until shipment. Ship on dry ice (-80°C).