Multiple Myeloma Banking Collection and Processing Protocol (WUSTL)

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

MM banking Collection and Processing Protocol

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PROTOCOL integer ID: 85927

Supplies

1

- ☐ 70% EtOH.
- ☐ PBS 1x (without Ca or Mg) [Fisher MT21-040CM]
- ☐ 0.5M EDTA ph 8.0 [Fisher NC9942732]
- ☐ Bovine Serum Albumin (BSA) [Sigma A2153]
- ☐ Fetal Bovine Serum (FBS) [Fisher SH3007103]
- ☐ Dimethyl Sulfoxide (DMSO) [Sigma D4540-100ml]
- ☐ Acridine Orange/Propidium Iodide (AO/PI) [Fisher NC0285242]
- ☐ Ficoll Paque Plus GE Healthcare [Fisher NC9778355]
- ☐ IMDM with L-Glutamine [Fisher MT10-016-CV]
- ☐ Liquid Nitrogen Cryotubes [MidSci TP89020]
- ☐ -196C Labels [MidSci LCRY-1700]
- ☐ 1.75ml Micro centrifuge tubes [MidSci MCT-175-B]

Reagents

2

- ☐ Freeze Media [DMSO/BS]: Thaw FBS at 37.9C. Add 45ml DMSO to 500ul FBS.
- ☐ Rinsing Buffer [EDTA/PBS]: Add 4ml of 0.5M EDTA liquid to 1000ml of PBS 1x.
- ☐ Running Buffer [BSA/EDTA/PBS] Reconstitute BSA to 5% in 110ml of sterile filtered water. Add 5% BSA solution and 4.4 ml of 0.5M EDTA liquid to 1000ml of PBS 1x.

All solutions should be combined in a hood for sterile purposes. Autoclaving all solutions before use is strongly recommended.

Equipment

3

- ☐ Pipettes and Micro Pipettes
- ☐ 15ml and 50ml conicals
- ☐ Microcentrifuge tubes
- ☐ Automacs cell separator
- ☐ Hemacytometer
- ☐ Microscope
- ☐ Centrifuge

□ -80C storage box
□ Isopropanol storage container
□ 4C Refrigerator
□ -80C Freezer
□ -196C Liquid Nitrogen Storage

Bone Morrow Isolation, iliac crest

4 Bone marrow aspirate (BMA) is removed from the iliac crest of a patient using a biopsy needle and placed in an EDTA Tube before the bone marrow mononuclear cells are isolated.

Bone Marrow Mononuclear Cells (BMMCs)

5 1. Add IMDM equal to the total BMA volume to the EDTA tubes.
2. In 15/50ml conicals, add BMA/IMDM mixture into each with an equal amount of Ficoll-Paque (i.e. total BMA/IMDM volume is 14ml, place 3.5ml Ficoll & 7ml BMA/IMDM into two conicals). Note, tilt the 15ml conical to a 45O angle and layer the BMA/IMDM on top of the Ficoll (Don't let the BMA mix with the Ficoll).
3. Centrifuge the 15/50ml conicals for 25 minutes with no brake at 1400rpm and 4C.
4. Carefully remove conicals from centrifuge and notice the layers. From bottom to top: RBCs, clear ficoll, cloudy interphase cells, and pink plasma + IMDM.
5. Using a glass pipette, collect the interphase cell layer avoiding Ficoll and place in a new 15ml conical.
6. Fill to the 14ml line on the conical with Rinsing Buffer and centrifuge 5min at 1500rpm and 20C.
7. After centrifuge, aspirate the supernatant and resuspend the pellet in 10ml of Rinsing Buffer.
8. Place a sterile pre-separation filter on top of a new 15ml conical.
9. Pre-wet the filter with 300ul of Rinsing Buffer, then filter the re-suspended cells.
10. Place 10ul of this re-suspension into a microcentrifuge tube.
11. Record total amount of resuspension before spinning.
12. Spin 15ml conical for 5min at 1500rpm and 20C.
13. In a 1ml cryovial, add 10ul AO/PI and 10ul aliquot of the BMMCs.
14. Place 10ul on hemocytometer and count live:dead, then record. Note, be sure to multiple by total volume of BMMCs
15. Remove supernatant, re-suspend in 1.5 mL of Freeze Media for approximately every 5E6 cells and aliquot into cryovials. Note, before use you must thaw Freeze Media in H2O bath.
16. Label each cryovial with barcoded label.
17. Store in cryovials of ~1e7 total cell aliquot(s).
18. Allow to Freeze slowly in a freezing chamber at -80□C for 24 hours, then place the vial in Liq Nitrogen for long term storage.
19. Complete the processing and sample documentation sheet(s).

6 Protocol Provided by DiPersio Lab and Vij Lab at Washington University.