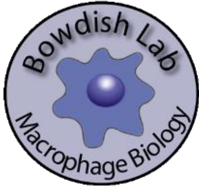

CULTURING, FREEZING, & DEFROSTING BONE MARROW-DERIVED MACROPHAGES

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BACKGROUND

- Following isolation of murine bone marrow, this protocol explains how to culture macrophage progenitors into bone marrow-derived macrophages to the point where they can be frozen down and subsequently defrosted for *in vitro* experiments.
- R10 medium with 15% L-cell conditioned medium (LCM) from fibroblasts is used to differentiate cells into bone marrow-derived macrophages.

NOTES

- All steps in this protocol should be performed in a BioSafety Level 2 (BSL2) laminar flow hood to maintain sterility. Gloves and a lab coat should be worn at all times.
- This protocol is for bone marrow flushed from 1 spine (i.e. 1 mouse). Adjust amount accordingly.

EQUIPMENT

- 150-mm Petri dish (Fisherbrand)
- 50mL conical tubes
- Pipette gun & 10mL, 25mL pipettes
- R10 media with 15% LCM
- Warmed Accutase
- Cell lifters
- Cryovials
- Pure FBS
- FBS with 20% DMSO

PROTOCOL

1. Following isolation of cells from bone marrow, resuspend cells in R10 medium containing 15% LCM. Bone marrow from one mouse can be resuspended in 5mL each.
2. Each plate should be prepared with 25mL R10 media + 15% LCM. Add 1mL of cell suspension per plate and swirl to distribute cells in medium. Therefore, for one mouse, you should have 5 plates.
3. Incubate at 37˚C and 5% CO_2. The cell culture process will take a week, allowing the Mø progenitors to attach to the plastic and divide until there is a confluent monolayer of Mø's. The media is specific for Mø's and all other cells present will die within two days of culture.
4. Feed Mø's on day 3 by adding 15mL R10 medium + 15% LCM.
5. Replace all media on day 6 with 25mL fresh R10 + 15% LCM.

6. On Day 7, your cells should be ready to harvest. Check under a microscope to ensure that cells have a spindle-like appearance, which signifies that they are macrophages.
7. Aspirate media from each plate.
8. Add 10 mL of accutase per plate and incubate at 37˚C for 10 minutes to allow the cells to lift off the plate.
9. Use a cell lifter to gently lift any remaining adhered cells.
10. Collect the cell suspension and add R10 medium to neutralize the accutase.
11. Spin down cell suspension for 5min at 1500rpm.
12. Resuspend cell pellet from each plate with 1 mL of pure FBS.
13. Add 0.5mL of cell suspension to a cryovial.
14. Add 0.5mL of 20% DMSO FBS in a dropwise fashion to each cryovial.
15. The vials were placed into a freezing container (Mr.Freezy) containing isopropanol at -80°C for 24 hours.
16. The frozen vials were then transferred to the liquid nitrogen tank at -140°C for long-term storage.

Defrosting BMDM

1. Warm media to 37˚C and add 15mL of RPMI media (without LCM) to 50mL conical tube.
2. Place cryovial in water bath (37˚C) until it thaws (approx. 1-2 min).
3. Pour thawed cell suspension into conical tube with warmed R10 media.
4. Spin down cell suspension for 5min at 1500rpm.
5. Resuspend with 10-25mL of warmed R10 (with 15% LCM) media and plate for future experiments.

Recipes for Media

RPMI-1640

- Remove 60 mL of pure RPMI media.
- Add FBS 50 mL.
- Penicillin/Streptomycin 1 vial (5 mL) - defrosted at 37°C, containing 10 000 U Penicillin/ml; 10 mg/ml Streptomycin
- 200 mM L-Glutamine 1 vial (5 mL)
- Add ~75 mL LCM if RPMI with 15% LCM is needed. [Refer to LCM protocol to know how to generate LCM].