Isolation and Culture of Mouse Bone Marrow-derived Macrophages (BMM'phi')

Ran Chen*

Department of Genetics, Stanford University, Stanford, USA
*For correspondence: rcchen@jfkbio.com

[Abstract] Bone marrow derived macrophages are a type of white blood cell that can be isolated from mammalian bone marrow. In this protocol, a method is described in which bone marrow cells are isolated from mouse leg bones (femur and tibia), and then differentiated to bone marrow-derived macrophages in approximately 10-day culture using L929-conditioned medium.

Materials and Reagents

1. L929 cells
2. RPMI 1640 medium (RPMI) (Life Technologies, Invitrogen™, catalog number: 11875-093)
3. Fetal bovine serum (FBS) (Atlanta Biologicals, catalog number: S10350)
4. Stock penicillin/streptomycin (P/S) (Life Technologies, Invitrogen™, catalog number: 15140-122)
5. DPBS (Life Technologies, Invitrogen™, catalog number: 14190-250)
6. 75% ethanol
7. Bone marrow growth medium (see Recipes)
8. BMM'phi' growth medium (see Recipes)

Equipment

1. Cell culture incubator
2. 0.22 μm filter
3. 27 g needle and 1 ml syringe
4. Scissors and forceps
5. 15 ml cell culture dish

Procedure

1. Preparation of L-cell conditioned medium: Culture L929 cells with initial 50% confluence in RPMI + 10% FBS for 5 days, collect the medium and filter with 0.22 μm filter, aliquot and store at -20 °C.

Note: Incubate FBS at 50 °C for 30 min before using.

2. Prepare bone marrow growth medium and BMM'phi' growth medium (see Recipes).
3. Isolation of mouse bone marrow cells.
   a. Sacrifice mouse and immerse mouse in 75% ethanol.
   b. Clip the skin mid-back and remove the skin from the lower part of the body.
   c. Remove tissue from legs with scissors and dissect away from body.
   d. Clean remaining tissue from the pelvic and femoral bones and separate at knee joint. Be careful not to break the bones. It is important to make sure that all the tissue is removed from the bones since cells associated with this can contaminate the marrow preparation and potentially overgrow the macrophages.
   e. Immerse the bones in 75% ethanol for 5 min, then immerse them in DPBS for 5 min and leave them in RPMI+P/S until the next step.
   f. Cut off each end of bone.
   g. Using a 27 g needle/1 ml syringe filled with bone marrow growth medium; expel the bone marrow from both ends of the bone with a jet of medium directed into a 15 ml cell culture dish.
   h. Change medium every 3 days. While in culture, some of the cells become attached, while many of them still grow in suspension, so spin-down and re-culture those cells in new dishes.
   i. After about 10 days, almost all cells become attached BMM'phi's, and then BMM'phi' growth medium is used for further culture and tests.

Recipes

1. Bone marrow growth medium
   a. Add 5 ml of P/S to a 500 ml bottle of RPMI. Remove 100 ml and save in a clean 500 ml bottle.
   b. Add 20 ml of heat-inactivated fetal calf serum and 40 ml of L-cell conditioned medium, and mix well.
2. BMM'phi' growth medium
   RPMI+10% FBS+10% L-cell conditioned medium

Acknowledgments

This work was funded by 5050 project by Hangzhou Hi-Tech District, Funding for Oversea Returnee by Hangzhou City, ZJ1000 project by Zhejiang Province. This protocol was

developed in the Cohen Lab, Department of Genetics, Stanford University, CA, USA [Chen *et al.* (unpublished)].

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