PROTOCOL FOR HARVESTING AND PLATING BONE MARROW-DERIVED MACROPHAGES

LIPID MAPS Protocol ID PP0000003500
Version 1, 10-24-06

MATERIALS AND REAGENTS

2 month old C57BL6 male mice
CO_2
Sterile DPBS
Sterile syringes, 10 mL
Sterile needles, 18 and 22 gauge
Sterile pipettes
Sterile 50 mL polypropylene conical centrifuge tubes
100 mm non-tissue culture treated Petri dishes (Fisher, cat# 08-757-13)
100 mm treated tissue culture Nunc dishes (Fisher, cat# 12-565-98)
Bone Marrow-derived Macrophage Growth Medium 1 (Protocol ID PS0000002900)
70% EtOH (ethanol)
Blunt end scissor (Fisher, cat# 13-806-2)
Mayo scissor (Fisher, cat# 13-804-6) or sharp dissecting scissor
Forceps (Fisher, cat# 08887)
Tissue culture hood
For the following procedure, use items that are sterile or have been sprayed with 70% EtOH

PROCEDURE

1. Immediately before surgery, sacrifice mice with CO_2.
2. Prepare one mouse at a time on a clean sheet of absorbent paper.
3. Spray all external areas of the mouse with 70% ethanol.
4. Using a blunt end scissor, make an incision 1 inch vertically from umbilical region to anterior region.
5. Extend this incision along the medial aspect of both rear appendages.
6. Gently pull the skin downward below the heels to expose the muscles, etc.
7. Using a sharp scissor, dissect tibias and femurs from surrounding muscles and tendons. Place the tibias and femurs into a 50 mL polypropylene tube containing 4˚C DPBS.
8. In a tissue culture hood, place the tibias and femurs on a non-tissue culture treated Petri dish and spray with 70% EtOH.

9. Using a sharp scissor, remove excess tissue, knee and heel.
10. With an 18 gauge needle, fill a 10 mL syringe with ~ 8.5 mL Bone Marrow-derived Macrophage Growth Medium (BMDMGM1; Protocol ID PS0000002900), then replace the 18 gauge with a 22 gauge needle. (Syringes may be prepared ahead of time)
11. Drill the needle into the end (previous knee junction) of the femur or into the end (previous ankle junction) of the tibia.
12. Flush 2 mL of the BMDMGM1 through the femur and another 2 mL through the tibia onto a new non-tissue culture treated Petri dish.
13. Suspend cells and medium 1 x with a 22 gauge needle and a 10 mL syringe.
14. Place the cells into a 50 mL polypropylene tube.
15. Divide the cells per mouse among 6 – 10 cm non-tissue culture treated Petri dishes and bring the volume to 7 mL with BMDMGM1 per dish.
16. Maintain cells in a humidified 37˚C incubator.
17. On day 4, wash the cells with 7 mL RPMI.
18. Add 7 mL fresh BMDMGM1 and place back in incubator.
19. On day 6, aspirate BMDMGM1, wash the cells 1 x with 5 mL 37˚C DPBS, add 5 mL of 37˚C DPBS plus 1 mM EDTA and incubate at 37˚C for ~ 5 minutes.
20. Pipette cells off the bottom of the dish using a 10 mL pipette and place in a 50 mL polypropylene tube containing 10 mL BMDMGM1.
21. Pellet the cells by spinning at 1500 RPM for 5 minutes in table top centrifuge.
22. Aspirate the supernatant and suspend cells in 1 mL fresh BMDMGM1 per mouse.
23. Count cells by making a 10 fold dilution (100 µL cell suspension plus 900 µL DPBS).
24. Plate cell density as outlined below in 37˚C BMDMGM1.
    6 well dish: 1×10^6 /3 mL medium
    60 mm dish: 3×10^6 /5 mL medium
    100 mm dish: 5×10^6 /7 mL medium
    150 cm^2 flask: 1×10^7/20 mL medium
25. Proceed to the protocol for Kdo_2-lipid A Treatment of Thioglycollate and Bone Marrow-derived Macrophages (Protocol ID PP0000001801).

Author: Donna Reichart
Date: 10-24-06