PROCEDURE PROTOCOL FOR HARVESTING AND PLATING BONE MARROW DERIVED MACROPHAGES FOR LIPID MAPS DISTRIBUTION

LIPID MAPS Procedure Protocol ID
Version 2, 5-12-09

MATERIALS AND REAGENTS

2-3 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
150 mm non-tissue culture treated Petri dishes (Falcon, cat# 351058)
100 mm treated tissue culture Nunc dishes (Fisher, cat# 12-565-98)
Bone Marrow Derived Macrophage Growth Medium 2 (PS0000003200)
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 in 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 of Bone Marrow Derived Macrophage Growth Medium 2 (PS0000003200), 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 Bone Marrow Derived Macrophage Growth Medium 2 (BMDMGM2) 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 10 mL syringe.
14. Place the cells into a 50 mL polypropylene tube.
15. Divide the cells per mouse among 2 – 150 mm non-tissue culture treated Petri dishes and bring the volume to 25 mL with BMDMGM2 per dish.
16. Maintain cells in a humidified 37˚C incubator.
17. On day 4, add 10 mL of fresh BMDMGM2 to existing medium and place back in incubator.
18. On day 6, aspirate BMDMGM2, wash the cells 1x with 5 mL of 37˚C DPBS plus 5 mM EDTA, add 10 mL of 37˚C DPBS plus 5 mM EDTA and incubate at 37˚C for ~ 15 minutes.
19. Pipette cells off the bottom of the dish using a 10 mL pipette and place in a 50 mL polypropylene tube containing 10 mL of BMDMGM2.
20. Pellet the cells by spinning at 2000 RPM for 5 minutes in table top centrifuge.
21. Aspirate the supernatant and suspend cells in 30 mL of fresh BMDMGM2 to wash and spin again at 2000 RPM for 5 minutes.
22. Aspirate the supernatant and suspend cells in 1 mL of BMDMGM2 per mouse.
23. Count cells by making a 10 fold dilution (100 uL cell suspension plus 900 uL DPBS).
24. Plate cell density as outlined below in 37˚C BMDMGM2.
    2 - 1 x 10^7 /10 mL BMDMG2/ 100 mm TC dish
25. Proceed to treatment protocol for Bone Marrow-derived Macrophages.

Author: Donna Reichart
Date: 5-12-09