HARVESTING AND PLATING THIOGLYCOLLATE ELICITED MACROPHAGES

LIPID MAPS Protocol ID PP0000001502
Version 3, 06-07-07
(Former title: Harvest and Plating Primary Macrophages)

MATERIALS AND REAGENTS

CO_2
Sterile DPBS
Sterile RBC lysis buffer (Fisher/eBioscience cat# 00-4333-57)
Sterile syringes, 5 mL
Sterile needles, 18, 22 and 25 gauge
Sterile pipettes
Sterile 50 mL conical centrifuge tubes
70% ethanol
Tissue culture hood

PROCEDURE

1. 4 days after injecting and immediately before harvesting the macrophages, 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. Cut a small incision below bellybutton (center of abdomen).
5. Gently rip the skin downward to expose intraperitoneal cavity.
6. Using a 5 mL syringe with an 18 gauge needle, withdraw 5 mL 4˚C DPBS and replace 18 gauge needle with a 25 gauge needle.
7. Inject 5 mL 4˚C DPBS into intraperitoneal cavity, being careful not to puncture any organ (liver, lung, etc) or intestine.
8. Repeat with another 5 mL 4˚C DPBS.
9. Carefully swish liquid around to pick up as many macrophages as possible from around the organs, etc.
10. Using a new 5 mL syringe with a 22 gauge needle, withdraw the macrophages from the intraperitoneal cavity, remove the needle and place the macrophage/DPBS suspension into a 50 mL conical centrifuge tube on ice.
11. Repeat withdrawal of macrophages.
12. Repeat steps 2-11 for each mouse.
13. Spin down macrophages/DPBS at 1500 rpm x 5 min at 4˚C. Save pellet.
14. Add 5 mL 4˚C RBC (red blood cell) lysis buffer to the pellet.

15. Suspend macrophages by gently pipeting up and down.
16. Incubate on ice for 15 min.
17. Spin down macrophages/RBC lysis buffer at 1500 rpm x 5 min at 4˚C. Save pellet.
18. Add 1 mL 37˚C RPMI 1640, 10% LIPID MAPS fetal bovine serum (FBS) and 1% Pen/Strep (Protocol ID PS0000001700), per mouse, to the pellet. (Be sure to follow PS0000001700 and not PS0000001701).
19. Suspend the macrophages by gently pipeting up and down.
20. Count cells by making a 10 fold dilution (100 µL cell suspension plus 900 µL DPBS).
21. Plate cell density as outlined below in 37˚C PMGM1 (Protocol ID PS0000001700):
    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
21. Proceed to Kdo_2 treatment protocol for Thioglycollate and Bone Marrow-Derived Macrophages (Protocol ID PP0000001801).

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Date: 6-7-07