HARVESTING AND PLATING PRIMARY MACROPHAGES

LIPID MAPS Protocol ID PP0000001501
Version 01, 1-18-06

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
100 mm plates (Fisher cat# 12-565-98)
60 mm plates (Fisher cat# 12-565-95)
6 well plates (Fisher cat# 07-200-80)
70% ethanol
Tissue culture hood

PROCEDURE

1. 3 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. Douse mouse belly with 70% ethanol.
4. Cut a small incision below bellybutton (center of abdomen).
5. Gently rip to reveal intraperitoneal cavity.
6. Using a 5 ml syringe with an 18 gauge needle, withdraw 5 ml of 4˚C DPBS and replace 18 gauge needle with a 25 gauge needle.
7. Inject 5 ml of 4˚C DPBS into intraperitoneal cavity being careful not to puncture any organ (liver, lung, etc.) or intestine.
8. Repeat with another 5 ml of 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, remove macrophages from the intraperitoneal cavity and place in a 50 ml conical centrifuge tube on ice.
11. Repeat removal of macrophages.
12. Repeat 2-11 above for each mouse.
13. Spin down macrophages/DPBS at 1500 rpm x 10 min at 4˚C. Save pellet.

14. Add 5 ml of 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 10 min at 4˚C. Save pellet.
18. Add 1 ml of 37˚C RPMI 1640, 10% LIPID MAPS FBS and 1% Pen/Strep (Primary macrophage growth medium 1, Solution protocol PS0000001700), per mouse, to the pellet.
19. Suspend the macrophages by gently pipeting up and down.
20. Count the cells and plate density as outlined below:
    100 mm plates: 2×10^7/10 ml medium
    60 mm plates: 6×10^6/ 5 ml medium
    6 well plates: 4×10^6/ 2 ml medium
21. Proceed to Treatment protocol for Primary Macrophages (PP0000001800).

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