

PROCEDURE PROTOCOL FOR HARVESTING THIOLYCOLLATE AND BIO-GEL ELICITED MACROPHAGES

LIPID MAPS Protocol ID PP0000001500
Version 1, 10-26-05

MATERIALS AND REAGENTS

CO₂
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. 3 days after injecting and immediately before harvesting the macrophages, sacrifice mice with CO₂.
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 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% LM serum and 1% Pen/Strep (Solution Protocol ID), 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
 - 12-well plates: 2×10^6 /1 ml medium
21. The bio-gel elicited macrophage yield will be approximately 50% less than the thioglycollate elicited.

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