PROCEDURE PROTOCOL FOR HARVESTING FOAM CELLS

LIPID MAPS Procedure Protocol PP0000003100
Version 1, 7-24-06

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
70% ethanol
Tissue culture hood

PROCEDURE

1. Prepare syringes before sacrificing mice. 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. Allow at least 2 syringes for injecting DPBS and 2 syringes for removal of DPBS/macrophages per mouse.
2. 4 days after injecting the thioglycollate into the intraperitoneal cavity (PP0000003000) and immediately before harvesting the macrophages, sacrifice mice with CO_2.
3. Prepare one mouse at a time on a clean sheet of absorbent paper.
4. Douse the belly with 70% ethanol.
5. Cut a small incision below bellybutton (center of abdomen).
6. Gently rip to reveal intraperitoneal cavity.
7. Carefully inject 5 ml of 4˚C DPBS into intraperitoneal cavity without puncturing any organ (liver, lung, etc.) or intestine.
8. Repeat with another 5 ml of 4˚C DPBS or until cavity is full of DPBS (approximately 7.5 ml). Be careful not to overfill and risk DPBS seeping out of cavity.
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 the DPBS containing the macrophages from the intraperitoneal cavity. Avoid fat in the intraperitoneal cavity.
11. Remove the needle from the syringe and place the macrophages in a 50 ml conical centrifuge tube on ice.

12. Repeat removal of DPBS/macrophages.
13. Repeat 2-12 above for each mouse. Optional-Inject an additional 5ml of 4˚C DPBS into the intraperitoneal cavity and repeat removal to retrieve as many macrophages as possible.
14. Spin down DPBS/macrophages at 1500 rpm x 5 min at 4˚C. Save the macrophage pellet.
15. Add 5 ml of 4˚C RBC (red blood cell) lysis buffer to the pellet. Suspend macrophages by gently pipeting up and down.
16. Incubate on ice for 15 min.
17. Spin down RBC lysis buffer/macrophages at 1500 rpm x 5 min at 4˚C. Save the macrophage pellet.
18. Add 1 ml of DPBS per mouse to the macrophage pellet.
19. Suspend the macrophages by gently pipeting up and down.
20. Remove 50 ul and dilute 1:20 in DPBS for counting.
21. Proceed to lipid extraction.

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