

PROCEDURE PROTOCOL FOR HARVESTING AND PLATING  
THIOGLYCOLLATE  
ELICITED MACROPHAGES FOR LM DISTRIBUTION

LIPID MAPS Procedure Protocol ID  
1-17-08

MATERIALS AND REAGENTS

CO<sub>2</sub>  
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  
150 mm TC plates  
70% ethanol  
Tissue culture hood

PROCEDURE

1. 4 days after injecting and immediately before harvesting the macrophages, sacrifice mice with CO<sub>2</sub>.
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 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, withdraw the macrophages from the intraperitoneal cavity, remove the needle and place the macrophages/DPBS suspension in a 50 ml conical centrifuge tube on ice.
11. Repeat withdrawal of macrophages.
12. Repeat 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 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 5 min at 4°C.  
Save pellet.
18. Add 1 ml of 37°C PMGM2 (PS0000003600) per mouse to the pellet.
19. Suspend the macrophages by gently pipeting up and down.
20. Count cells by making a 10 fold dilution (100ul cell suspension plus 900ul DPBS).
21. Plate cell density as outlined below in 37°C PMGM2 (PS0000003600):  
150 mm plate:  $2 \times 10^7$ /20 ml medium

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