Arming Pulmonary Macrophages for Pathogen Defense
Program Project

SOP for Tissue harvesting and cell preparation for flow cytometric isolation
Version 1.0

I. Objective of the SOP

The objective of this document is to provide detailed guidelines for preparation of cells for flow cytometry sorting, beginning with dissection of tissues.

II. Version History

v1.0 Prepared by Amariliz Rivera *et al*. This version is our current stable as of April 2015.

III. Methods

1. Mice are anesthetized with ~300 µl of avertin solution (tribromoethanol 10mg/mL), and exsanguinated.
    a. Avertin solution
        i. 20g 2,2,2- tribromoethanol
        ii. 20 mL 2-methyl-2-butanol
        iii. dd$H_2$0 to 1L
2. Lungs are perfused with 10 mL of 1X PBS using a 10 mL luer-lock syringe with a
    a. 27 ½ gauge needle.
3. Lungs are extracted from mouse, and placed in a collagenase IV solution (Worthington 43N14578).
    a. Collagenase solution
        i. 0.071 g of collagenase IV is diluted in 40 mL of 1X PBS.
            1. 5 mL of collagenase solution needed per set of lungs.
        ii. Solution is filtered through a 0.45 µm sterile filter unit PVDF membrane using a 60 mL syringe before use.
4. 1 mL of collagenase solution is placed in a 60mm x 15mm sterile polystyrene plate (1/set of lungs)
5. Lungs are placed in 1 mL of the solution on the plate, and finely minced using a razor blade.
6. 4 mL of collagenase is added for a total of 5 mL of collagenase solution used per set of lungs.
7. Lungs are incubated at 37°C at 5% CO_2 for 45 minutes.
8. Single cell suspension is made by passing the lung several times through a 5 mL luer lock syringe and 20 gauge needle.
9. The single cell suspension is then filtered by placing a funnel lined with 100 µm nylon mesh filter paper on top of a new 15 mL conical tube.
10. Centrifuge lung homogenate at 1500 rpm for 5 min.
11. Discard supernatant.
12. Resuspend pellet in 3 mL of Red Blood Cell Lysis Buffer.
    a. RBC Lysis Buffer pH 7.2-7.4
        i. NH_4CL (155mM)
        ii. $NaHCO_3$ (10 mM)
13. After proper resuspension, add 5 mL of 1X PBS to neutralize RBC Lysis Buffer
14. Centrifuge lung homogenate at 1500 rpm for 5 min.
15. Discard supernatant.

16. Resuspend pellet in 1 mL of 1X PBS and count.
17. Centrifuge lung homogenate at 1500 rpm for 5 min.
18. Discard supernatant
19. Resuspend pellet at 10 million cells/ mL of FACS Buffer (0.1% sodium azide in 1XPBS) containing Fc Block.
20. Incubate on ice for ~20 min mildly agitating by hand every few minutes to prevent sedimentation of the cells.
21. Fill tube with FACS Buffer and centrifuge sample at 1500 rpm for 5 min.
22. Discard supernatant
23. Resuspend in FACS staining panel (see below)
24. Incubate on ice for ~60 min mildly agitating by hand every few minutes to prevent sedimentation of the cells.
25. Fill tube with FACS Buffer and centrifuge sample at 1500 rpm for 5 min.
26. Resuspend pellet at 10 million cells/ mL in 1% FBS in 1X PBS.
27. Add DAPI to sample.
    a. DAPI stock is diluted to 5 mg/ mL
    b. 1 µl/ mL is added to sample.
28. Sort (see SOP for sample collection).