

SOP-P034

Ammonium Chloride Lysing of Whole Blood

Objective: To isolate white blood cells including neutrophils, from whole blood for further immunological studies such as immunophenotyping, kinetic assays, or membrane potential.

Procedure:

1. Using a serological pipet transfer 2ml of whole blood to a 50cc centrifuge tube.
2. Add 48ml of working lysing reagent. See SOP#-P017 for preparation of working lyse reagent.
3. Securely tighten the lid onto the 50cc tube.
4. Place the tube on a rocker for 10 minutes at room temperature.
5. Centrifuge at 400xg for 10 minutes.
7. Decant supernatant and place the 50cc tube upside down onto a couple of layers of paper towel.
8. Swab the inside of the tube carefully with a guaze 4 x 4 held by hemostats. Do not disturb the cell pellet.
9. Run the tube across a test tube rack to break up the pellet.
10. Add 10ml of washing buffer (Phospate Buffered Saline). See SOP#-P020 for preparation of PBS.
11. Centrifuge for 10 minutes at 200xg.
12. Decant supernatant and run tube across rack. Add 10ml PBS (or other wash buffer).
13. Centrifuge 10 minutes at 200xg.
14. Decant and rack as before.
13. Resuspend the cells in a known volume of PBS.
14. Do a cell count on the Coulter Counter. See SOP#P005 for operation of the Coulter Counter.
15. Adjust to desired cell concentration.

Created by: Kathy Ragheb **DATE:** 2/24/99

Verified by: _____ **Date:** _____

Print Name

Sign Name