Creighton UNIVERSITY

# Complement Depletion of T-Cells

(Greg A. Perry, Ph.D.)

## Equipment:

15ml Conical tubes
Pasteur pipettes

## Reagents:

Hanks Balanced Salt Solution (HBSS)
Cell preparation
Monoclonal anti-Thy-1 antibody (clone HO13.4)
Baby Rabbit Complement (Pel-Freeze #31061-3)
RF10 (or RF10M) media
Lympholyte-M (Cedarlane #CL5031)

## Method:

1. Make cell preparation to a concentration of $3x10^7$ cells/ml in HBSS with calcium.
2. Add the HO13.4 monoclonal antibody to the cells, for a final concentration of approximately 5 mg/ml.
3. Incubate 40 minutes at $4^oC$.
4. Centrifuge and wash cells in HBSS w/ $Ca^{++}$.
5. Centrifuge and resuspend cells to $3x10^7$ cells/ml in HBSS w/ $Ca^{++}$.
6. Add baby rabbit complement to cells in a 1:16 ratio (1 part complement to 15 parts cells).
7. Incubate 30 minutes at $37^oC$.
8. Centrifuge and wash cells in HBSS w/ $Ca^{++}$.
9. Centrifuge and resuspend cells in 3-5 ml of HBSS w/ $Ca^{++}$ (approximately $1x10^7$ cells/ml).
10. Underlayer with an equal volume of Lympholyte-M (up to 5ml) using a Pasteur pipette.
11. Centrifuge 20 minutes at 1250g at room temperature.
12. Remove viable lymphocytes from the Lympholyte-M / media interface.
13. Add 3ml of RF10 to cells.
14. Centrifuge and wash cells in 5ml RF10.
15. Centrifuge and wash cells again in 5ml RF10.
16. Resuspend cells in small volume of RF10 and count.