Lee Lab 8/14/04

Protocol for Counting BMMs Cindy Rohde 1/7/04

- 1. Aspirate media from well using a 200 μL pipet tip.
- 2. Wash cells 3x with 1 mL ice-cold PBS.
- 3. Add appropriate volume (~300 μL for a 24-well plate) of ice-cold PBS/0.005% Zwittergent to each well.
- 4. Incubate plate at 4°C for 10-15 min.
- 5. Use a 1 mL pipteman to detach cells from plate.
- 6. Check that most cells are detached under the microscope.
- 7. Use a 1 mL pipetman to resuspend cells and remove a 100 µL aliquot.
- 8. Mix $100 \,\mu\text{L}$ aliquot with $20 \,\mu\text{L}$ trypan blue and transfer to hemacytometer.
- 9. Count cells.