Creighton
UNIVERSITY

Standard Technique for Mouse Cell Preparations

(Greg A. Perry, Ph.D.)

(Note: Can be used for Spleen, Thymus, Lymph Node, Peyer's Patch, and others)

Equipment:

Mouse
Needles
Sterile Scissors
15ml Conical tubes (2 per tissue)
60mm Petri dish
Pipettes
1cc Syringe
Ice bucket & ice

Reagents:

70% Ethanol
Sterile media (HBSS or culture media)
ACT (Ammonium Chloride Tris) lysis solution
Sterile media with serum (5% or more serum is fine)

Methods:

1) Sacrifice mouse by cervical dislocation.
2) Wet fur with 70% ethanol.
3) Clip fur and remove tissue of interest to 60mm Petri dish containing sterile media. Keep on ice.
4) Remove cells from the tissue by:
    a. Teasing apart the tissue using needles.
    b. Aspirate the tissue up and down using a 1cc syringe until most of the cells are removed from the tissue.
5) Decant the cells (and tissue ghost) into a 15 ml conical tube and let sit on ice for 5 minutes. (Note: This lets the remaining tissue fragments settle out.)
6) Remove the supernatant into a clean 15ml conical tube.
7) Centrifuge for 10 minutes at 350g (approx. 1000-2000 rpm).
8) Discard supernatant.

Tissues containing a significant number of red blood cells (such as spleen, blood and bone marrow) must be shock-lysed prior to doing cell counts. Tissues without RBC's (such as Thymus, lymph node and Peyer's patch) do not need shock-lysing. For these tissues, proceed directly to step 13.

9) Resuspend cell pellet in 5ml of ACT and allow to sit on ice for 5 minutes.
10) Add 5ml of serum containing media (example: PBS+5%FBS).
11) Centrifuge for 10 minutes @ 350g (approx. 1000-2000 rpm).
12) Discard supernatant.
13) Resuspend cell pellet in known volume of media for cell counts.

General guidelines on final volume:		
	Spleen	1-2 ml (depending on size)
	Thumus	1 ml
	Lymph Node	1 ml
	Peyer's' Patch	1 ml