University of Michigan Comprehensive Cancer Center	SOP-BCR-3.1	**Cell Counting**	Author: S. Clouthier Approved: M. Wicha	Rev: 1.0	Issued: 09/24/98 Revised: 7/13/12

1.0 Purpose

Cell counting is done in order to determine an accurate number of viable cells in a given cell suspension.

2.0 Scope

Quantification of cells is necessary in order to set up FACS analysis, determine accurate bone marrow dosages and to analyze differences between mice.

3.0 Description

An aliquot of cell suspension is diluted (typically 1:4) in Trypan blue dye and acetic acid. Dead cells take up the dye and appear blue under a light microscope. Acetic acid acts to lyse RBC's. There is some amount of nonspecific lysing of WBC's as well; therefore the cells should not be allowed to remain in the acid for long periods of time prior to counting. If RBC's are not present, acetic acid may be omitted from the dye mixture.

4.0 Materials and Reagents

No.	Name	Description	Concentration	Storage Location
1.0	Acetic Acid	Glacial Acetic acid	4%	026-328S
2.0	Trypan Blue	Trypan blue/ PBS	1:3 dilution of 0.4% Trypan blue in 1X PBS	026-328S

5.0 Procedure

5.1 Aliquot 1X ul of cell suspension into a 96 well U bottom plate (typically 40ul).

5.2 Add 1X 4.0% acetic acid (typically 40ul) to the well. Sample will turn clear.

5.3 Add 2X Trypan Blue (typically 80ul) to the well and mix by pipetting.

5.4 Place 10ul of diluted, stained cells on both sides of a clean hemacytometer. Count the number of non-blue cells. Count between 30-100 cells. Readjust cell suspension volume if too few or too many cells are present. Attempt to count only the large, single membrane lymphocytes. Exclude counting the smaller double membrane RBC's. If replicate counts vary by more than 10%, continue counting cells until an accurate cell count is achieved.
In order to determine total # of cells, use the following equation:
Average # cells counted x dilution factor (e.g. 4 for 1:4 dilution) x multiplication factor used to convert hemacytometer well depth to ml (10^4) = 10^6 cells/ml x ml = total # cells.

5.5 Record cell counts and determine cell concentration.

6.0 Reference(s)

Not Applicable.

7.0 Procedural Change Description

Rev Level	Date	Reference	Description of Change
1.0	7/13/12	CL	Updated room locations