Cell Number Determination

1. Count total number of cells in the 4 sets of 16 squares on haemacytometer
2. Take the average number of cells for the 4 sets of 16 squares

Concentration of cells = average obtained in step 2 **x** how many times it is diluted **x** 10^4 cells / mL

Total number of cells in whatever amount we have resuspended all the cells in
= Concentration of cells **x** volume

Cell Viability Determination

The dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, Eosin, or propidium, whereas dead cells do not. In this test, a cell suspension is simply mixed with dye and then visually examined to determine whether cells take up or exclude dye. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm.

Materials

- PBS *or* serum-free complete medium
- 0.4% trypan blue (store in dark bottle and filter after prolonged storage; GIBCO/BRL)

1. Centrifuge an aliquot of cell suspension being tested for viability 5 min at 100 × *g* and discard supernatant.

   The size of the aliquot depends on the approximate number of cells present. The aliquot should contain a convenient number of cells to count in a hemacytometer when suspended in 1 ml PBS and then diluted again by mixing with 0.4% trypan blue (e.g., 5 × 10^5 cells/ml).

2. Resuspend the cell pellet in 1 ml PBS or serum-free complete medium.

   Serum proteins stain with trypan blue and can produce misleading results. Determinations must be made in serum-free solution.

3. Mix 1 part of 0.4% trypan blue and 1 part cell suspension (dilution of cells). Allow mixture to incubate ⍰3 min at room temperature.

> *Cells should be counted within 3 to 5 min of mixing with trypan blue, as longer incubation periods will lead to cell death and reduced viability counts.*
>
> *Mixing can be performed in a well of a microtiter plate or a small plastic tube using 10 to 20 µl each of cell suspension and trypan blue.*

4. Apply a drop of the trypan blue/cell mixture to a hemacytometer (*APPENDIX* 3A). Place the hemacytometer on the stage of a binocular microscope and focus on the cells.

5. Count the unstained (viable) and stained (nonviable) cells separately in the hemacytometer. To obtain the total number of viable cells per ml of aliquot, multiply the total number of viable cells by 2 (the dilution factor for trypan blue). To obtain the total number of cells per ml of aliquot, add up the total number of viable and nonviable cells and multiply by 2.

6. Calculate the percentage of viable cells as follows:

% viability = (unstained cells/total cells) **x** 100 %

% dead cell = (stained cells/total cells) **x** 100 %

Strober, W. (2001). Trypan Blue Exclusion Test of Cell Viability. Current Protocols in Immunology, Appendix 3:Appendix 3B. doi: 10.1002/0471142735.ima03bs21