Counting Cells

Preparing cells to be counted (Adherent cells in a 10 cm plate)

1. Warm cell media and trypsin
2. Aspirate cell media
3. Wash cells with 5 mL of PBS 3 times
4. Add 3 mL of trypsin to plate
5. Place cells in incubator until cells are rounded and/or floating
6. Use a P1000 set to 1000µL to wash cells down off plate while it is tilted. Rotate plate to ensure all cells are washed off the plate
7. Add 9 mL of appropriate cell media to the cell plate
8. Pipet this mixture up and down with 10 mL pipet and transfer cells to a 50 mL centrifuge tube
9. Centrifuge cells for 5 minutes at 1000 rpm at RT
10. Remove supernatant from the 50 mL centrifuge tube being careful not to disturb the cell pellet
11. Add 10 mL of appropriate cell media to the 50 mL centrifuge tube and pipet up an down to resuspend the cell pellet
12. Using a P1000 pipet, add 0.5 mL of this cell suspension to a sterile 1.5 mL Eppendorf tube
13. Using a P200 pipet, add 0.4 mL of 0.4% trypan blue to the Eppendorf tube from the previous step and mix gently
    - REF: 15250-061
    - This stains dead cells so information about the cells viability can be collected as well

Counting Cells

1. Clean hemocytometer and glass cover slip with 70% ethanol. More information on the hemocytometer on the following page.
2. Moisten the coverslip with water and affix to hemocytometer
3. Gently mix cell trypan blue mixture created and add 100 µL evenly between both chambers of the hemocytometer
4. Using a cell counter (something that you click and the number on the device increases), count the number of viable cells is the 4 corners and center section of the grid. Do this for both sides of the hemocytometer (Make sure to keep numbers from both sides of the hemocytometer separate)
    - When looking at one of the 3x3 sections of the grid that is to be counted, only count the cells that are within this section or on the right-hand or bottom boundary line
    - If you are trying to look at viability information than you can count the number of blue cells (dead cells stained by trypan blue)

Interpreting the Data

At this point, there should be 20 numbers that are needed for further calculations, one for each grid square that cells were counted in it. Make sure you keep numbers from the different sides of the hemocytometer separate. The values calculated below can be averaged at the end, but values from both sides should be compared to make sure they are similar before averaging.

The sum of all cells per side should be taken first to get the total number of viable cells. If dead cells were counted as well, the same can be done to get the number of total nonviable cells:

$$\text{Total Viable Cells} = \sum_{i=1}^{5} x_i \quad i \text{ is each grid square and } x_i \text{ is the num of viable cells in grid square } i$$

$$\text{Total Nonviable Cells} = \sum_{i=1}^{5} x_i \quad i \text{ is each grid square and } x_i \text{ is the number of nonviable cells in grid square } i$$

The percentage of viable cells can be calculated as:

$$\frac{\text{Total Viable Cells}}{\text{Total Nonviable Cells}} * 100$$

The average number of viable cells per square:

$$\frac{\text{Total Viable Cells}}{\text{Grid Squares}}$$

Cell dilution factor (in our case the dilution factor is 5):

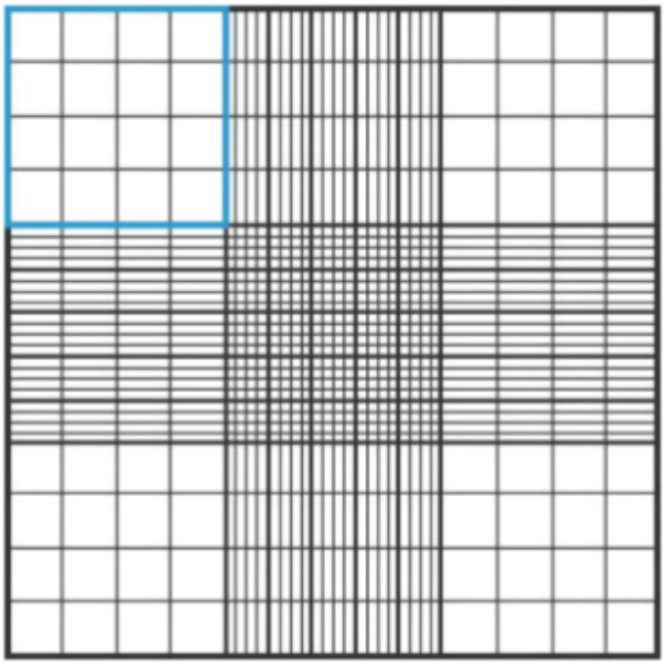
$$\frac{\text{Total Volume of Original Trypan/Cell Mixture}}{\text{Volume of Cells added to Trypan Cell Mixture}}$$

Concentration (viable cells/mL):

$$\text{Average number of viable cells/square} * \text{Dilution factor} * 10^4$$

Hemocytometer General Information

Above is a picture of the hemocytometer. The glass coverslip should be centered over the metal part when placed on the hemocytometer. The hemocytometer is split up into two sections which are delineated by the indent separating the two. The trypan/cell mixture is added to the half-oval on each side of the hemocytometer. Each of these sections has a grid on it as show below:



Each of the 9 sections of this grid contain 10^4 mL of liquid. People count different number of sections on the grid, but this protocol will ask you to count 5; all corners and the center section.