cellgro® by Mediatech, Inc.

Trypan Blue

Trypan blue is the most common stain used to distinguish viable cells from nonviable cells. Viable cells absorb Trypan blue over time, affecting counting and viability results. Live, healthy cells appear round and refractile without absorbing the blue-colored dye. Conversely only non-viable cells absorb the dye and appear blue, and also may appear to be asymmetrical. The use of this stain, however, is time-sensitive. Make dilutions just prior to counting to prevent viable cells from absorbing the stain, and thus appearing non-viable.

In addition, trypan blue has a greater affinity for serum proteins than for cellular proteins to properly view and count the cell. For cells cultured in high serum conditions, the background in the hemacytometer may be too dark. In this case, cells must be isolated from the serum. Simply centrifuge the cells and resuspend the cell pellet in a balanced salt solution or serum-free media prior to counting.

Procedure

1. Prepare a uniform cell suspension of the culture to be counted.

2. Dilute 0.4% (w/v) Trypan Blue 1:2 (1:1). Add 0.4 ml of the diluted Trypan Blue to small disposable test tubes. Add 0.1 ml cells to make a 1:5 dilution, or add 0.2 ml cells to make a 1:3 dilution, or add 0.4 ml cells to make a 1:2 dilution.

3. Center a cover glass over the hemacytometer chambers. Fill one chamber with the cell dilutions using a pipette. The solution will pass under the cover glass by capillary action. Do not overfill. If the solution spreads into the two lateral grooved adjoining the grid table, clean the hemacytometer and repeat the application. If there are any bubbles in the solution covering the grid table, clean the hemacytometer and repeat the application.

4. Place the hemacytometer on the stage of an inverted microscope and adjust focus using 100X magnification.

5. Use a hand-held counter to record cell counts in each of the four corner and central squares. Five squares (four corner and one center) are counted for a total of five squares. See figure 1.

6. Determine the number of cells per milliliter and total number of cells using the following calculations:

$$\textbf{cells/ml} = \frac{\textbf{\#of cells counted}}{\textbf{\# squares counted}} \times 10^4 \times \textbf{dilution factor}$$

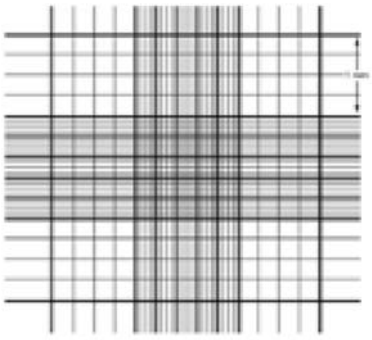
$$\textbf{total cells} = \textbf{cell/ml} \times \textbf{vol. of original cell suspension}$$

7. The percentage of viable cells can also be calculated using using the following formula:

$$\%\textbf{viability} = \frac{\textbf{\#viable cells counted}}{\textbf{total \# cells counted}} \times 100$$

Important: Count cells touching the middle line of the triple line of the top an left of the squares. Do not count cells touching the middle line of the triple lines on the bottom or right side of the square.

Figure 1.
Improved Neubauer Hemacytometer Counting Grid



	Catalogue No.	Size
Trypan Blue 0.4% (w/v) in normal saline	MT 25-900-CI	6 x 100mL