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Instructions: Manual Enumeration of Cells

For counting human and animal blood and bone marrow derived cells

1. Prepare a 1:10, 1:20 or 1:200 dilution of cells in 3% [0.5 M] acetic acid in a tube or a well of the 96 well plate (for a 1:10 dilution dispense 10 µL of the sample into 90 µL of acetic acid).
2. Prepare the same dilution as step one in trypan blue in a tube or a well of the 96 well plate.
3. Clean the hemocytometer and place the cover slip over the gridded areas.
4. Dispense approximately 5-7 µL of the acetic acid cell suspension onto one side of the hemacytometer. One the other side of the hemocytometer place 5-7 µL of the trypan blue cell suspension and let stand for approximately 20 seconds.
5. Place the hemocytometer on the light microscope.
6. Starting with the acetic acid cell suspension, enumerate the cells within a certain number of squares until a minimum of 100 cells are counted.[1]
7. Enumerate the cells in the trypan blue cell suspension in the same number of squares as in step 6. In addition, determine the number of white cells and blue cells in the trypan blue cell suspension.
8. Compare the total cell counts between the two squares – this will determine if accurate pipetting was performed in the generation of the cell suspensions. If the total cell numbers are within 15% of each other, proceed with the cell count determination. If the variability in cell number is greater than 15%, start the procedure again.
9. To determine the cell count, the following equation can be used:[2]

$$\frac{cells}{ml} = (average\ cells\ per\ square)(dilution\ factor, i.e.\ 10, if\ 1{:}10\ was\ used)(10^4)$$

10. To determine the percent of viable cells in the sample, divide the white cell number from the trypan blue count by the total cell number (blue and white) and multiply by 100%.

ReachBio cannot guarantee cell count accuracy using alternate procedures.

MANUAL CELL COUNTING should be performed for cell count accuracy. Cell counting using Automation devices, FACS counting of cells via propidium iodide staining, and other methods are **NOT ACCURATE** and are not recommended.

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[1] If more than 100 cells are counted in one large square, the cell suspension is too concentrated and will need to be diluted to get an accurate cell count. If less than 50 cells are enumerated in the total of 9 large squares, the cell suspension is not concentrated enough to get an accurate cell count and a different dilution needs to be prepared).
[2] Each of the 9 squares of the hemacytometer with cover slip in place represents 0.1 mm^3 (or 10^{-4} cm^3) which is equivalent to 10^{-4} mL.

ReachBio recommends the use of manual enumeration when using single cell or purified populations.