CELL COUNTING QUICK REFERENCE GUIDE

OVERVIEW

Counting of cells when using the LeviCell™ system requires counting at 3 different points in the workflow: **1)** during sample preparation to achieve desired cell input concentration, **2)** for accurate sample input cell number and viability, and **3)** for output yield and viability . The recommended cell counting method uses AO-PI (Acridine Orange and Propidium Iodide) and a Nexcelom automated counter if available for consistency and more reliable counts. Levitas can provide a Nexcelom cell counter for certain demos if needed (and depending on availability).

Cell counting, viability, and viable cell yield are the most common evaluation criteria in LeviCell demos, and these guidelines must be followed in order to achieve reliable counts:

A. Stock Concentration

1. Cells in their stock buffer/media should be counted (using dilutions if needed) to obtain an accurate concentration. Consult your automated cell counter recommendations for optimal cell concentration, and dilute the stock cell suspension as needed to achieve the recommended optimal concentration.
    a. This measurement is used to calculate the volume of stock cells required for the desired total cell input (live + dead) within a range of 250K to 1M cells into the LeviCell cartridge. Use the spreadsheet 90-00016_A_Demo_Data_Collection_Template to calculate the volume of stock cells to pellet.
    b. Pellet cells at 300 RCF for 5 minutes, and then carefully remove supernatant without disturbing the cell pellet.
        1. If a cell pellet is not clearly visible, it is OK to leave 10-20µL of cell buffer volume in the bottom of the tube to ensure the cell pellet is not aspirated.

B. Input Sample Concentration

1. Prepare 300µL of Levitation Buffer by adding the appropriate volume of Levitation Agent (LA) (1M stock) to 1X PBS + 0.5% BSA (e.g. for 150mM final LA, use 45µL LA + 255µL PBS-BSA).
    a. NOTE: 1X PBS + 0.5% BSA is the preferred buffer to use with LeviCell. Substitutions can be made depending on the unique requirements of the cells.
2. Add 270µL of Levitation Buffer (LB) to the cell pellet to resuspend the cells. Pipette gently 5-10X to thoroughly mix the cells.
    a. *Note:* If cell counting requires >30µL, add the entire 300µL of LB prepared in step 2
    b. *Note:* For the most accurate counts, two independent measurements should be made of the input sample.
3. Before loading the LeviCell cartridge, take an aliquot of resuspended cells for counting.
    a. Set aside for counting immediately after loading the required 220µL into the LeviCell cartridge.

b. This measurement is required to calculate the ACTUAL total cell input to the LeviCell cartridge. This is a CRITICAL measurement as cell loss during the cell pelleting process is guaranteed and accurate cell input and viability counts are essential for comparing viable cell output, cell viability, and yield metrics post-enrichment with the LeviCell system.

C. Output Yield and Viability

a. After levitation is complete, cells from the Top channel (viable cells) should be counted immediately after recovery from the LeviCell cartridge (follow the cell/volume recovery process described in the Harvesting Cells QRG)

b. If cell recovery volume is too low to use the desired volume for counting, take a smaller volume (no less than 2µL) and bring up to the volume required for counting. Make sure to note the dilution performed when making final cell count calculations.