Cell Counting

Last Revision: June 13, 2017

Version: 1.0

Written by: Anne Marie Barrette

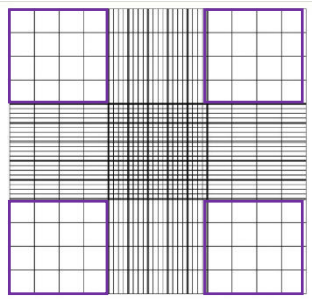
Validated by / Date:

This protocol describes how to obtain cell count from adherent or suspension cells using a hemocytometer. Accurate cell counts are critical to nearly every aspect of cell culture assays.

1. Prepare Reagents
    a. Heat the appropriate media and cell detachment solution (e.g. trypsin) in a 37°C water bath until the temperature is equilibrated (~10 min—see Subculture protocols).
2. Prepare Hemocytometer
    a. Clean glass hemocytometer and coverslip with 70% ethanol and wipe with a Kimwipe.
    b. Once dry, place the cover slip over the reflective portion of the hemocytometer that contains the counting grid, leaving a small portion of the v-shaped groove exposed for sample delivery.
3. Prepare Adherent Cells
    a. Aspirate media from cells and add PBS to coat the flask.
    b. After gentle rocking/swirling of the PBS, aspirate PBS from flask and add appropriate amount of cell detachment solution for the specific cell line (see Subculture Protocol).
    c. Return flask to incubator and check for detachment of cells after 5 minutes and every 5 minutes after that if necessary.
    d. Return flask to tissue culture hood and add enough of the warmed media to bring the cell volume up to 10 mL (for a T75 flask—scale as appropriate), pipetting up and down along the bottom of the flask (where cells were attached) to ensure that all cells are successfully detached.

e. Collect the cell-containing media from the flask and transfer to a sterile 15 mL Eppendorf tube and spin at the appropriate time and speed to pellet the cells at RT (typically 100g for 5 min, but see Subculture Protocol).
f. Under the sterile hood, aspirate media from the 15 mL tube, being careful not to disturb the cell pellet.
g. Resuspend the cell pellet in 10 mL of fresh media (for a T75 flask—scale accordingly) by pipetting up and down, making sure cells are evenly distributed and no clumps remain.

4. Preparing Suspension Cells
    a. Collect cell-containing media from flask and transfer to a 15 mL Eppendorf tube and spin at the appropriate time and speed to pellet the cells (typically 100g for 5 min, but see Subculture protocol).
    b. Aspirate media from the 15 mL tube and resuspend cell pellet in 10 ml of fresh media (for a T75 flask), making sure cells are evenly distributed and no clumps remain.
5. Count Cells
    a. Remove 10 µL of cell-containing media from the 15 mL Eppendorf tube with a sterile micropipette, and transfer into the hemocytometer chamber underneath the coverslip
        i. Pipette slowly, allowing the cell suspension to be drawn out by capillary action.
        ii. Note here we have not instructed addition of Trypan Blue staining solution as is often done. Trypan Blue is used to estimate % viability, as viable cells exclude Trypan Blue. If viability is thought to be an important factor in the cell count at hand, then add 10 uL of Trypan Blue (CAT #15250061) to the 10 uL of cells, and add this to the hemocytometer. Remember to multiply by an additional factor of two below (Step 6) if this is done.



    b. Place the hemocytometer on an inverted microscope and focus the grid lines with a 10X objective in phase contrast.
        i. You will see a 3x3 grid with the 4 corners containing 4x4 separations (i.e. 16 smaller squares). These are the 4 squares from which you will be counting purple outlines in the image to the right).
    c. Using a hand tally counter, count the cells present in one of the purple squares.
    d. Move the hemocytometer under the microscope repeat for the three remaining purple squares.
6. Calculate Cell Concentration
    a. Divide the total number of cells counted by four.
    b. Multiply that number by 10,000 to get the number of cells per mL.
    c. Multiply by the total number of mL of cell suspension (i.e. 10 mL for the T75 as above) to get the total number of cells present in the sample.
7. Cleanup
    a. Spray the hemocytometer and the cover slip with 70% ethanol, wipe with a Kimwipe and return the hemocytometer and cover slip to their storage box.