

S2 Cell Culture

Cell Culture

Materials:

To a 500 ml bottle of Schneider's Media, add 55.5ml IFS (inactivated fetal calf serum) to a final concentration of 10% and 5 ml Penicillin-Streptomycin to a final concentration of 1U/ml pen./ 1ug/ml strep.

Serum Free Media: Add 10 ml Glutamine upon opening to 1 Liter.

T75 flasks

Pipettes

70% ethanol in a spray bottle

The cells:

We are using a *Drosophila* Schneider 2 cell line that grows very quickly and is easily maintained. Cells double about every 24 hours. They are grown in Schneider's media supplemented with fetal calf serum and antibiotics and maintained at 25° C or lower. Stocks will be grown in T75 flasks and will be split about every 3 days. Experiments will be done in 6 well plates.

Sterile Technique:

All cell culture must be performed in the tissue culture hood, also known as a biological safety cabinet. The hoods protect the samples from aerosol contamination by filtering all of the air that enters. In addition, to prevent other contamination, spray down all the inside working surfaces of the hood with 70% ethanol and wipe the surfaces dry with kimwipes before and after using the hood. It is also a good idea to spray down items brought into the hood from the outside such as pipettors and media bottles. Set up all the materials you may need in the hood before bringing in the cells such as the media and flasks, but also try not to clutter the hood with unnecessary items. They may get in your way. In general, work quickly with good sterile technique to avoid contamination.

Procedure:

Volume: Start culture at 25 million cells in 12 ml media in T75 flask.

Duration: Grow cultures for ~3 days and then split.

- 1.) Tap the flask to loosen cells from the surface. If cells are not resuspended well by tapping, remove cells with a scraper. These cells are not very adherent, so they will lift off the surface fairly easily.
- 2.) Pipet up and down 10X to create a single cell suspension
- 3.) Count # of cells with a hemacytometer
- 4.) Resuspend cells to appropriate density and seed in flasks.

Counting cells and assaying cell survival by Trypan Blue staining:

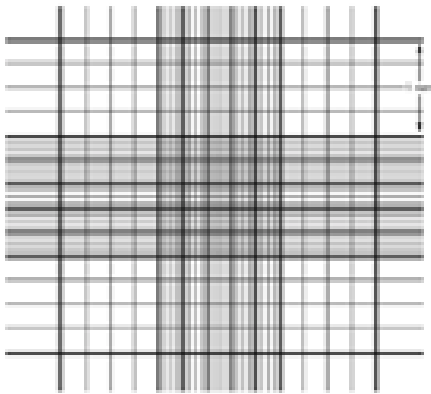
Materials:

Neubauer hemacytometers

Trypan Blue diluted to 0.8 mM in PBS. Store at room temperature. Stable for 1 month.

Hand held counters

Fig1: A hemacytometer counting grid



Procedure:

1. Prepare a uniform cell suspension as described above. Suggestion: dilute cells 1:5 or 1:10 before counting. Dilute in media, and take an initial aliquot of cells no smaller than 200 ul (a smaller aliquot may be less uniform).
2. Mix a small aliquot of cells 1:1 with trypan blue solution. Note: when counting cells for normal passaging, trypan blue is not needed. Viable cells exclude trypan blue, while dead cells stain blue due to uptake of the dye. However, this assay does not distinguish necrotic vs. apoptotic cells.
3. Center a cover glass over the hemacytometer chambers. Fill one chamber with the cells using a Pasteur pipette. The solution will enter the chamber by capillary action. Do not overfill. If the cells spreads into the two lateral grooves adjoining the grid table, clean the hemacytometer and repeat the application.
4. With an inverted microscope, count the cells located in each of the four corners and the central squares of the hemacytometer, for a total of five squares. See fig. 1. Using the 20X objective with the 10/20 filter (the very left one) produces the best visualization of the cells. Use a hand-held counter to record the number of cells counted. During the trypan blue exclusion assay, don't worry about calculating percentage of viable cells. Simply generate total live cell count by only counting circular white cells, and not the blue cells. The total cell count should exceed 100 to be reliable. If after counting five squares, 100 cells have not yet been counted, then count additional squares.
5. Determine the number of cells/ml and the total number of cells using the following formula:

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

total # cells = cells/ml x vol. of original cell suspension

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

$$\% \text{ viability} = \frac{\# \text{ Viable cells counted}}{\text{Total \# cells counted}} \times 100$$

7. Wash cells off the hemacytometer with water. Spray some 70% EtOH and let dry.

Notes

- Avoid prolonged exposure of cells to trypan blue since uptake of the stain is time-sensitive. If exposure exceeds 30 minutes, some viable cells may have absorbed the stain, resulting in an increase in trypan blue-positive cells and yielding an artificially higher dead cell population.