

Subcloning Hybridoma Cells (for Monoclonal Antibody Production)

I. Cell Concentration Determination.

1. Pipette cells in well up and down to make uniform mixture of cells. Remove some with pipette and place 15 μ l into an eppendorf tube.
2. Add 5 μ l 0.25% trypan blue to the eppendorf and mix with the cell suspension. Drop this mixture into the crevice of the hemocytometer, below the coverslip. Add just enough to cover the rectangular metal surface.
3. Using the low power objective, focus on the hemocytometer grid. Change to the medium power objective to focus on each of the four quadrants of the grid.
4. Live cells will appear bright and yellowish clear against the blue background. Count the number of live cells in each quadrant.
5. Apply the following formula:

$$((\text{Total live cells}/4 \text{ quadrants})/3/4)/0.0001 = \# \text{ Cells/ml}$$

(3/4 is the factor by which the cells were diluted by the trypan blue)

II. Subcloning

1. Label three 96-well tissue culture plates with all pertinent information. Designate which one is to be the undiluted, 1:4, and 1:16 plates.
2. Put the volume of cells which represent 1500 cells into a 50 ml Falcon tube. Add enough warm hybridoma media to make the total 28 ml. (Hybridoma media contains 500 ml Gibco BRL Opti-Mem media, 50 ml FCS, and 4 ml 100X Glutamine. HAT selection is optional at this point)
3. Using a 5 ml pipette, aliquot 0.2 ml diluted cells into each well.
4. When a plate is filled, dilute the remaining cells 1:4 and aliquot as before.
5. Repeat step 4.
6. Put all plate in incubator at 37°C and 5% CO₂. Check for colonies in 7 to 10 days.