Plating Efficiency

Adapted from Mather, J.P., and P.E. Roberts, 1998. Introduction to Cell and Tissue Culture: Theory and Technique. Plenum Press. New York and London.

A plating efficiency is a measure of the number of colonies originating from single cells. It is a very sensitive test and is often used for determining the nutritional requirements of cells, testing serum lots, measuring the effects of growth factors, and toxicity testing.

Materials

1. Cells
2. Cell culture plates or flasks
3. Centrifuge tubes
4. Growth medium
5. Trypsin
6. 10% formalin
7. 0.1% crystal violet

Procedure

1. Trypsinize the cells (see Passage of Adherent Cell Lines, Page 102) and centrifuge the cells.
2. Resuspend in 5–10 mL or growth medium and count the cell suspension (see Hemocytometer Counting).
3. Calculate the volume of the suspension that would be required to achieve concentrations of 2, 10, and 20 cells/cm^2 of surface area.
4. Plate the appropriate volume of suspension in duplicate culture plates/flasks. It is important to mix the suspension prior to plating to ensure an even suspension of cells.
5. Incubate the cells for approximately 10 days. This time will vary depending on the cell line and the conditions. The colonies should be visible with the naked eye but should not be joining together.
6. Wash the plates/flasks with PBS, cover the cells with 10% formalin and fix for 10 min.
7. Remove the formalin, add crystal violet to cover the cells and let sit 10 min.
8. Rinse with water until no additional color comes off the flask/plate.
9. Count the colonies and calculate a plating efficiency. Plating efficiency = number of colonies formed/number of cells plated x 100.