Appendix IX

TRYPSINIZATION AND MAINTENANCE OF MONOLAYER CELL CULTURES

Monolayer cell cultures may be kept active and available for seeding of tubes, vials, dishes and plates. A constant supply of cell cultures can be maintained by routine subpassage of cell lines to new flasks. (Generally research use only.)

1. Discard medium from the cell culture flask (125 cm^2). Rinse monolayer with 15 mL of Hank's Balanced Salt Solution and discard.

2. Add 5 mL of trypsin EDTA mixture (pre-warmed to 36^oC) to flask.

3. Incubate the culture flask for 3 minutes (no more than 5 minutes) at 36^oC. Observe after 3 minutes to see whether the cell sheet is breaking loose from the flask surface. Tap flask sharply against palm of hand to aid in loosening tissue.

4. When tissue has loosened completely, add 15 mL of pre-warmed growth medium into the flask.

5. Mix cells by drawing cells and fluid up and down in a pipette.

6. Adjust the volume to 90 mL with growth medium.

7. Aliquot 30 mL of suspended cell mixture into at least one new 125 cm^2 culture flask. Aliquot the remaining to shell vials at 1.5 mL each or tube culture (16 x 125 mm) at 2 mL each.

8. Incubate the culture flasks, shell vials or tube cultures at 36^oC.

9. Observe daily for growth of cells (3 - 5 days) and for change in pH of medium. If the medium becomes acidic or basic, replace it with fresh growth medium.

10. Replace growth medium with maintenance medium when a confluent monolayer is obtained (usually after 2 - 3 days).

11. Replace with fresh maintenance medium once a week.

Note: Freeze first passage of cell culture whenever a new shipment is received. (See Appendix VIII).