Cell Culture and HeLa
Manual
NAME: _____ Week 9 Lab

Background

From the readings, you should be able to:

- describe the origins and basic properties of 3T3 and C2C12 cell lines
- visualize confluence
- be comfortable working with scientific notation in the context of cell density

Objective

To experience working with cells in culture to gain appreciation for the importance of HeLa cells for medicine and medical research.

Activities

How these tasks are divided will depend on how well-prepared people are (having read and are familiar with every step of the procedure) and student interest.

Active participation may take the form of reading instructions, passing solutions and materials to classmates, wiping things down, labeling culture dishes as well as working in the hood or with the tasks below. Scribe is an essential task as everything must be written down in the TC lab notebook.

All tubes and culture dishes taken from sterile packages must be handled with sterile gloves
All media removed from the water bath must be sprayed down prior to use in the hood.

Part 1: Preparations

1. Remove trypsin, PBS and culture media needed from 4°C and place into 37°C water bath, allowing to warm prior to use.

2. Turn on microscope and Countess

3. For each cell type, label a 12-well culture dishes with the date, the cell type and the letter of each condition at the correct well.

4. Determine who is doing what tasks.

Part 2: Removing cells from T-75 flask

1. Observe cells at 40X and note % confluence in cell notebook

2. Using a 10 ml pipet, remove all media

3. Add 5 ml 1X PBS and swish flask back and forth to remove residual media

4. Add 1 ml Trypsin-EDTA and swish

5. Return flask to incubator for 3-5 minutes

6. Tap flask firmly on the cell side and observe cells, returning to incubator if necessary

7. All 4 ml DMEM+10% FBS to flask and wash cells to bottom

8. Transfer cells and media to 15 ml conical tube

Part 3: Determining density

1. Remove 7.5 µl cells and transfer to a 1.5 ml Eppendorf tube

2. Add 7.5 µl trypan blue, pipet to mix

3. Let sit 1 minute

4. Transfer cells and dye to one side of a Countess hemocytometer

5. Carefully insert into the Countess, observe and collect data

6. Record information in lab notebook

7. Given this density, how many cells will you be plating in each well if you use 500 µl of cell per well?

Part 4: Plating under different conditions

1. While cell density was being calculated, 1 ml of appropriate media was added to each well of a 12-well TC plate

2. Add 500 µl of cell to each well

Has everything been written down in the TC lab notebook?

Have you determined a schedule of when students will be observing the cells between Tuesday and Friday or Thursday and Monday? These students need to coordinate with Dr. Pisano