Procedure for Splitting Cells for Regrowth and Testing

Tools and Equipment:  
new cell culture flask

centrifuge

1ml centrifuge vials

pipette with 10ml graduated tips

bleach

phosphate buffer solution (PBS)

cell food (10% FBS PFS solution)

vial rack for the centrifuge vials

50ml sealable test tube

Personal Safety Equipment:

For bio safety level 2, use of hood, gloves, and lab coat for person protection. With the hood shield down, protective eye ware was not needed. The hood should be turned on, and the shield lowered so only your hands can fit underneath it, before any work with the cells is done. The cells are not to leave the bio safety level 2 lab room.

Notes:

The cell suspension will become more acidic over time, and thus, the more yellow or orange than dark pink (the color of the cell food).

The cells are being kept under conditions of 37 d C and 4.9% CO2 in the incubation chamber when not in use.

The food is being stored in a standard refrigerator in a bio safety lab room.

The process does not need to leave the room to be completed.

Procedure

Centrifuging:

1) Remove the flask from the incubation unit and place it in the hood

2) Using the pipettes, put 1 ml of cell solution from the cell culture flask into each of the centrifuge vials until no more solution in the flask. 10 vials should be full.

3) Seal the vials, and evenly distributed them in a centrifuge which is then run at 1.7krpm for 5 min. Once complete, there will be a pellet of cells in the bottom of those vials. If there is an odd number of vials, do not use the odd one, place it aside on the rack for later disposal.

4) Split the vials into two equal groups. One group will be used to create the solution for testing and the other will be used to regrow the cell population.

For Cell Regrowth

1) Siphon off the solution in the vials and put it in the test tube.

2) Using a new pipette, add 1ml of food to the vials. The tip of the pipette will not touch the cells.

3) Shake the vials to dissolve the pellet.

4) Using a pipette, add the solution of cells and cell food to a new cell culture flask. The flask should be labeled with the type of cells or strain, and what path number they are on.

5) The flask is then sealed, and placed horizontally back in the incubation chamber until this process needs to be repeated.

Preparing Cells for Testing

1) Repeat step 1 from the Cell regrowth section

2) Using the pipette, add 1 ml of a buffer solution, phosphate saline buffer solution is added to the vials

3) Repeat step 3 of the Cell Regrowth section

4) Using the pipette, the solution can be added as needed to the test fixture. It can also be stored temporarily in test tube or a cell culture flask.

For unused centrifuge vials

1) Repeat step 1 from the Cell regrowth section

2) Using the pipette, add 1ml of bleach to the vials

3) Shake the vials to dissolve

11) Using a pipette, the bleach and cell solution is added to the mixture of old cells already in the disposable test tube.

Clean up:

If the disposable test tube has not had bleach added to it, several milliliters should be added to it. The liquid should turn light blue to clear, and now can safely be disposed. All surfaces in the hood and materials that are not disposable should be wiped down with 70% ether solution. All equipment used that is disposable should disposed of in bio-safe bins which should be located near the hood.