HOST-PATHOGEN INTERACTIONS LAB
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TITLE: FREEZING OF HELA CELLS:

Date: 15/07/2021

Name: Ipsita Nandi

Reagents/Materials required

1. Freezing medium containing 10% DMSO (Thermo Scientific Fisher, Cat#12648010)
2. Complete Growth Medium (DMEM with 10% FCS, 1% Gln, 1% PSA) for HeLa
3. Trypsin C (Sartorius, # 03-053-1B).
4. 1x PBS (Sartorius, #02-023-1A)
5. 1-2ml Cryovials (Thermo scientific Fisher, Nunc, #2025-06)

Things to be Ready:

1. Warm 1xPBS, Growth Medium, Freezing medium and Trypsin
2. Marked labels

Procedure

1. First, register in the Tissue culture logbook and in the inventory log in PC by writing the vial number, name of cell, source of cell, your name, date, place in the liquid nitrogen tank.
2. Print stickers in DYMO by typing all the details of the cells to be frozen (For example: Name of the cell, date, your initial, source, number of the vial etc.).
3. Prepare 2 Cryovials per one cultured cell plate (10 cm; sterile in the Tissue culture) by placing caps above it and marking the vial number of the cell in the logbook, and label the vials with the printed stickers ***(Do not start the steps in Tissue Culture unless all the above-mentioned steps are ready)**
4. Aspirate Growth medium.
5. Add 10ml of 1xPBS to the plate (for washing).
6. Aspirate 1x PBS. Repeat steps 5 and 6 and aspirate PBS as much as possible.
7. Add 1ml of Trypsin C to the plate
8. Place in the CO_2 incubator at 37°C for 3min. Tap the plate several times and see if most cells are dissociated. If not, incubate the plate at the CO2 incubator for additional 1-3 min (don't over trypsinize, as over trypsinization may hamper the cells).
9. Add 5-10ml of growth medium and resuspend the cells by up and down pipetting, using a 10-ml pipette. Collect the resuspended cells in a sterile 11ml conical tube.

FREEZING OF HELA CELLS:

10. Spin down at 1500 rpm for 1.5min.
11. Aspirate the medium and suspend the cell pellet by finger-flipping in minimal volume.
12. Add 2ml of freezing medium to the tube and resuspend cells by pipetting up and down using a 1-ml or 2-ml pipette.
13. Add 1ml of freezing medium with cells to each of the two previously prepared cryo-vials.
14. Close vials tightly and place in thermocol box (designed for gradual slow freezing of the cells at -70°C).
15. Carry the box immediately to Revco#1(-80°C) and keep it there for up to 4 days, and not longer. Then, transfer vials to liquid nitrogen tank for storage.