SOP-P039

Thawing HL60 Cells from the Cell Freezer

Objective: To use the HL60 cells for experiments

Products: T75 Flask, Non Treated, angled CS100 (VWR cat#89092-700)
2ml pipettes, polystyrene, non-plugged, individually wrapped, sterile (VWR cat# 53106-450)
25ml pipettes, Polystyrene, Plugged, sterile, individually wrapped (VWR cat#89130-900)

Procedure:

Thawing Medium: RPMI 1640, no Glutamine (Life Technologies, cat#21870-100)
10% FBS Heat Inactivated (Life Technologies, cat#10082-147)

1. Look in the log book for the cells location and passage number you want. Using the thermal gloves, carefully open the cell freezer lid and remove the rack which contains the cells you need. Lifting the side rods, remove the box that contains your cells. Open the lid of the box carefully not to disturb the other vials and remove the vial labeled with the type and passage you want to use.

2. Put the lid back on the box, place in rack, replace the metal rods and put the rack back in the cell freezer. In the log book, record your initials and day that you removed the vial.

4. Place the vial in the 37°C water bath. You want to thaw cells rapidly. Label the flask (T75, non TC) with cell type, passage number, date, and your initials. As soon as they are thawed, wipe off the top of the vial with ethanol. Place in the hood.

5. If the cells were frozen using 10% DMSO, you have to dilute them with a 1:20 dilution. (Add 500 uls of medium to the vial.) If 5% DMSO, use a 1:10 dilution. (Add 1ml of medium to the vial.)

6. Add the vial amount to the T75 flask containing 35mls of fresh medium. Add some of the medium back in to the empty vial and pipette up and down gently to remove the remaining cells. Add this volume back in to the T75 flask.

7. Since this type of flask has the cap vented, be sure the cap is closed completely. Place the flask in the 37°C for a week or so minimum.

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Verified by:_____Date: 4/1/2013

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