SOP-P004

Freezing HL-60 Cell Cultures

Objective: To prepare cultured HL-60 cells for long-term storage in liquid nitrogen (LN_2)

Procedure:

1. Culture enough cells to provide 5-10x10^6 cells/ml (refer to SOP#-XXX for HL-60 Cell Culture).
2. In a sterile environment such as a laminar flow hood, gently aspirate the cell culture with a sterile pipet to mix and transfer the suspension to one or more sterile centrifuge tubes. Cap the tube(s).
3. Spin down the cells at room temperature for 6 minutes at 250xg in a Beckman GS-6R centrifuge. The centrifuge is located in the Bindley building, room 133.
4. In a sterile environment, remove the supernatant and resuspend the cell pellet in cold RPMI 5/5, using half the required volume (i.e. resuspend at 10-20x10^6 cells/ml).
5. For each ml cell, label two sterile 2 ml freezing vials with contents, passage number, date, and your initials.
6. Aliquot 0.5 ml cell suspension per vial and place vials on ice.
7. To each vial, slowly add 0.5 ml sterile RPMI 5/5 containing 10% DMSO and cap the vials. This cryopreservative solution is cytotoxic, so freeze the cells immediately.
8. Put the vials in a Nalgeneä Cryo 1°C freezing container supplied with 250 ml isopropyl alcohol (as indicated on the container) and place the container in the -80°C freezer for at least 4 hours. This freezer is located in the Bindley building, room 133.
9. Within 48 hours, transfer the frozen cells to the liquid nitrogen freezer. The LN_2 freezer is located in the Bindley building, room 133. Record the storage location in the freezer logbook.

Created by: Gretchen Lawler February 12, 1999

Verified by:_____ Date:_____ _____

Print Name Sign Name