SOP-E049 (ray)

AMGEN Automation of HL60 Assay

Cell Culture:

BIOLOGICAL SAFETY CABINET STERILIZATION SETUP
UV is turned on for and allowed to run for 15 minutes. The hood is then started (UV bulb will not stay on with hood motor), and allowed to run for 15 minutes. Surfaces are sanitized with 70% EtOH and cell culture work can commence.

Note: Robotic hood is started at the same time as the 222 b/c BSC

ROBOT STERILIZATION SETUP
UV is turned on for and allowed to run for 15 minutes. The hood is then started, with UV bulb still on, and allowed to run for 15 minutes.

BULK STOCK
Previously HL60 cells were bulk frozen at 1.0 x 10^7 cells / ml in RMPI-1640 + 10 % FBSc + 15 mM HEPES + 10% DMSO (plant cell grade) in Nanopure water [filter sterilized at 0.2 um], frozen and stored at -80 degrees C.

THAWING
Prewarm RMPI-1640 + 10 % FBSc medium at 37 degrees C. After the medium is warm, place HL60 bulk frozen cell into 37 degrees C water bath to thaw. When thaw is about 50% complete add an add equal volume of RMPI-1640 + 10 % FBSc medium to finish thawing. Gently transfer into roller bottles at 2.0 x 10^5 cells / ml (or lower dilution depending on cell need timing) and place into Bellco Roller Bottle Appartus (37 degrees C / 1 RPM) for growth. The doubling time of HL-60 is between 36 – 48 hours, and presently it appears to be closer to 36 hours.

DO NOT CENTRIFUGE / DO NOT ROUGH DISPENSE as these operations appear to change cryo-trauma into cryo-necrosis.

GROWTH
Allow growth to continue to ~ 1.0 x 10^8 cells / ml. After cell counting is accomplished 400 mls of roller bottle cell stocks are withdrawn and cells are centrifuged at 250 x G for 5 minutes to pellet the HL-60 cells. 100 mls of remaining cell stock is centrifuged at replated into new 500 mls of medium for appropriate cell density for the required growth period (tentatively 2.0 x 10^5 cells / ml for 3 days growth, 1.0 x 10^5 cells / ml for 6 days growth).

ROBOTIC CELL STOCK
The HL-60 cells are suspended in RMPI-1640 + 10 % FBSc to make a cell suspension for robotic dispersion at 5.0 x 10^6 cells / ml. For 4 plates, 20 mls of 5.0 x 10^6 cells / ml in RMPI-1640 + 10 % FBSc for a final volume of 80 mls is made. A pregating control of 20 mls of RMPI-1640 + 10 % FBSc for the hypercyt setup.

Both tubes are placed in the 222D CO2 incubator until dispensed by Screening lab personnel prior to Chemical exposure and subsequent staining.

MEDIUM
RMPI-1640 + 10 % FBSc

Automation:

Format is set: Every second row: 10 conc of dye, duplicates, 21, 22 are DMSO controls, 23, 24 not used
Volumes: cells 20ul of 1 x 10E7 (20,000 cells total per well)
Chemicals: 20 ul of 2x conc

Assay:

1. Chemicals loaded day prior to assay - in 96 well plate, 3 fold serial dilutions in medium (DMEM)
2. Load to 384 well plate, stored frozen, seal with tape
3. Take chemical loaded plate. Add cells
4. Incubate 6 hours
5. Spin down plate 250 x g 5 min
6. Remove soup robot - 30 ul removed of total of 40 (75% removal)
7. Add 40ul dyes JC1 - 5 uM; Valinomycin last 4 wells for JC-1
   Calcein 30,000 diln of 1 mg/ml (.03ug/ml) ; mBBr 40uM ; Mitosox 10uM ?????

   DYES are made in PBS in 0.1% BSA (Mitosox is in DMSO)

8. Cover with tape
9. Spin plate 250 x g 30 seconds (to remove bubbles)
10. Shake 10 seconds @ 2200 RPM

Cells and dyes in bulk given to flow for instrument setup. Sent to flow

Flow Cytometry:

1. Instrument must be on 30 mins prior to receiving samples
2. Incubate plates 10 min @ 37 deg (for dye incubation)
3. Bulk cells used to set up cytometer:
4. 2 ml for JC-1 dyed - split ??????
5. Cells - 1 hour prior to end of 6 hour incubation flow will do setup controls

Flow Cytometer setup and operation:

1. Set up flow protocols
   The protocol is shown in Appendix / JC1 / Redox

Hypercyte setup and operation:

1. Set up prewritten protocols: JC1 and Redox
2. 30 second prime, 30 second shake @3000 RPM, run
3. Run REDOX first (rows a, c, etc) / Save and covert data to Hypercyte / Wash probe 1 minute, rinse 1 minutes
4. Run JC1 protocol / Save and covert data to Hypercyte / Wash probe 1 minute, rinse 1 minutes

Data Processing & Archiving:

Run hypercyte analysis

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Verified by:____Date:_7/11/2012_________

Print Name Sign Name