SOP-E050 (Larisa)

AMGEN Automation of HL60 Assay

Cell Culture:

1. HL60 cells - log cells are bulk frozen and stored
2. Prewarm 37 deg buffers (TCPBS in naopure water), plus 0.1% BSA final), 0.2um filters
3. 50 ml conical tube of cells 1 x 10E7/ml thawed to 50% thaw, add equal volume of RPMI to accelerate thaw, place into 37 deg incubator

1. Medium from 4 deg to 37 deg 10 min
2. Cell counted usually Ok
3. Sanitize robot and hood (15 minutes)

Medium: DMEM

Automation:

1. Format for 384 well plate is set:
2. Columns ##2-20, chemical compounds at 9 concentrations, uM,: 300, 100, 33.33, 11.11, 3.70, 1.23, 0.41, 0.137, 0.046.
3. Columns 1,2,21-24, controls: no compounds,.
4. 2 dye panels, panel 1: rows A,C,E, G, I, K, M, O, panel 2 – rows B, D, F, H, J, L, N, P
5. Duplicates per each dye,
6. Volumes: cells 20ul of 1 x 10E7 (20,000 cells total per well)
7. Chemicals: 20 ul of 2x conc

Assay:

Biomek FX robot

1. Chemicals prepared same day as assay - in 96 well plate, 3 fold serial dilutions in medium (RMPI-1640 + 10 % FBSc), 100 ul of 2xconcentrations of chemicals.
2. Transfer chemicals to 384-well plate (20 ul/well).
   Biomek300 (inside Biosafety hood)
3. Add cells (20 ul per well).
4. Incubate 6 hours at 37C, CO2 incubator.
5. Spin down plate 250 x g 5 min.
6. Aspirate supernatant - ~30 ul removed of total of 40 (75% removal).
7. Add 40ul dye Viability/Redox panel to rows A,C,E, G, I, K, M, O;
8. Add 40ul dye JC1 to rows B, D, F, H, J, L, N, P; Valinomycin is added to last 4 wells for JC-1(welLs P21-P24).
9. Cover with tape and spin plate 250g x 30 seconds (to remove bubbles).
10. Shake 10 seconds @ 2200 RPM.
11. Sent to flow (to place to incubator at 37C for 10 min)

    Dyes are made in PBS in 0.1% BSA Calcein 30,000 dilution of 1 mg/ml, final 0.03ug/ml ; mBBr , stock 40mM, final 40 uM, Mito-sox 10mM in DMSO, final 10uM.
    JC-1 stock 5mM, final 5 uM, valinomycin 1000 dilution of stock.

Cells and dyes in bulk given to flow for instrument setup

Flow Cytometry:

Instrument must be on 30 mins prior to receiving samples

Incubate plates 10 min @ 37 deg (for dye incubation)
Bulk cells used to set up cytometer:

2 ml for JC-1 dyed - split ??????
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:
Set up prewritten procols:
JC1
Redox

30 second prime, 30 second shake @3000 RPM, run

1. Run REDOX first (rows a, c, etc) / Save and covert data to Hypercyte
2. Wash probe 1 minute, rinse 1 minutes
3. Run JC1 protocol / Save and covert data to Hypercyte
4. 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