

## SOP-P056

### Amgen HL60 - Cell Culture - Automation

#### Objective: To Culture HL60's Assay

#### Cell Culture:

HL60 cells - log cells are bulk frozen and stored  
Prewarm 37 deg buffers (TCPBS in naopure water), plus 0.1% BSA final), 0.2um filters  
50 ml conical tube of cells  $1 \times 10^7$ /ml thawed to 50% thaw, add equal volume of RPMI to accelerate thaw, place into 37 deg incubator

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

Medium: DMEM

#### 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  $1 \times 10^7$  (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)

1. Load to 384 well plate, stored frozen, seal with tape

Take chemical loaded plate

Add cells

Incubate 6 hours

Spin down plate 250 x g 5 min

remove soup robot - 30 ul removed of total of 40 (75% removal)

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)

9. Cover with tape

Spin plate 250 x g 30 seconds (to remove bubbles)

Shake 10 seconds @ 2200 RPM

Cells and dyes in bulk given to flow for instrument setup

Sent to flow

#### Dye Solutions:

**JC-1 Dye** (quantity for preparing one 384 well plate)

1. Stock concentration for JC1 is 5mM in DMSO

2. prepare in ratio of 1µl of stock / 1ml of PBS with 0.1%BSA

3. Thaw one aliquoted (9µl) vial of JC1 from freezer. Add this to 9mls of PBSw/0.1% BSA.

Mix well, cover with foil.

**Redox Dye** (quantity for preparing one 384 well plate)

1. Stock concentrations  
CalceinAM: 1 mM. in DMSO  
mBBr: 40 mM in DMSO  
Mito-sox: 50ug
2. **Mito-sox:** To each of two 50ug vials of mitosox, add 6.58µl of DMSO, Mix well.
3. **mBBr:** Take one aliquot out of freezer and thaw.
4. **CalceinAM:** Add 1µl of stock to 30mls of PBS /0.1% BSA , Mix well
5. Transfer 9mls of the Calcein AM dilution into a 15cc conical tube
6. To this 15cc tube, add 9µl of Mito-sox and 9µl of mBBr, mix, cover with foil.
7. Label as "Redox Staining Solution".

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**Created by:** Kathy Ragheb

**Verified by:** \_\_\_\_\_ **Date:** 6/1/2012 \_\_\_\_\_  
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