SOP-E005

Amgen HL60 – Flow Cytometry – Cyan/ Hypercyt

Objective: To run Amgen HL60 plates on the Cyan/ Hypercyte

Flow Cytometry

CyAn must warm up for 30 mins prior to running samples.

Run flowcheck QC beads to check instrument

Run beads for fluorescence calibration and make sure they are coming within a couple of channels of the set position(mean) for each PMT.

Beads	PMT(dye)	means
Level II	calcein(FL1-log)	13
	Mitosox(FL4-lin)	76
	mBBR (FL6-log)	124
Level III	calcein(FL1-log)	71
	Mitosox(FL4-lin)	off scale high
	mBBr(FL6-log)	230
	JC-1_525(FL1-log)	32
	JC-1_590(FL2-log)	275

Incubate plates for 10 mins @ 37° (for dye incubation) in the incubator.
Bulk cells are used to set up the cytometer. Use leftover cells and dye from the robot to set up instrument controls.
From the bulk cells, remove 1ml each for the following controls: cells only, redox, and JC1.
Spin down for 10 mins @ 300xg. Remove the supernatant, resuspend the pellet.
Add 1ml of the appropriate dyes to the tubes. Split the JC1 tube in half and add 1μl of valinomycin to one of the tubes.
Incubate the control cells in a 37° water bath for 10 mins. Run on the cytometer.

Cells - 1 hour prior of the 6 hour incubation, flow will do the instrument setup controls.

After the instrument control cells have been run, hook the pump tubing up to the CyAn and put the half 12x75mm test tube on the sample station and close the lever.

Prime the tubing and check that the segments look good.

HyperCyt Summit Automator

Click on the Hypercyt summit automator icon from the desktop
Click on set-up
Go to the same save path that you have set in the summit software
Click on start
In Hypercyt Controller click on the **reset** button
DO NOT CLOSE THE AUTOMATOR BOX, just click anywhere else and it will minimize

Flow Cytometer setup and operation:

Redox
1. Open up redox protocol
2. The protocol is shown in Appendix
3. The Hypercyt system will start the CyAn acquisition
4. Save the fcs file when the plate has finished running (20100303_redox_p00001a)

JC1
1. Open up the JC1 protocol
2. The protocol is shown in Appendix
3. The Hypercyt system will start the CyAn acquisition
4. Save the fcs file when the plate has finished running (2010303_JC1_p0001a)

Hypercyte setup and operation:

Prime the instrument and check that it is producing good segments, no bubbles, consistent length.

Run the redox experiment first, then run the JC1, being careful to rinse well after to flush out the valinomycin.

In **HyperView Designer** make a new experiment and name it with the date and either an r or j at the end.
Experiment name should be in this format: **20100303r (YYYYMMDD)**
Set up the following parameters for the experiment in Designer:

Pre-plate 60 second (up-down prime), 30 second shake @3000 RPM, up time= 0.5sec., sip time = 1.0sec.
Pump speed= 18.0rpm, interwell shake @3000RPM for 2 seconds, after every 24 wells

Label the plate using the following format: **p0001a**

Save experiment

Open **Hypercyt Controller**
Find your experiment and open it
Select current plate
Make sure the plate is properly seated on the shaker platform

Click on Hypercyt Controller **RUN** button

Run REDOX first (rows a, c, etc)
Wash probe 1 minute in S3, 2% contrad, rinse 1 minute at S1, 0.1%BSA/PBS
Run JC1 protocol
Wash probe 1 minute, rinse 1 minute

Summarize Proper Labeling - **IS VERY IMPORTANT:**
Experiment name: 20100303r (or j) (YYYYMMDD)
Plate name: p0001a
Analysis name: AM1r (or j)

Data Processing & Archiving:
Open Hyperview Analysis

See SOPp066 for Hypercyt_Redox and JC1 Data Analysis

After all plates have been analyzed in HyperviewAnalysis and files have been transferred to Jumble, then go on to the drug analysis, on the other computer in HyperCyt room:

Click on ICON on desktop: Cyan folder on jumble/template/plate check.xls/format.

This opens in format tab
Enter plate names > protocol tabs

Work thru tabs across the bottom:

1. raw redox/file/open/.cyan shortcut/cyan data folder on jumble
2. find date> select STAT file>open
3. highlight row A thru F > control C > tab plate check.xls.
4. make sure you are on correct tab (raw redox)
5. highlight A1 box in plate check > click > control V (paste)
6. redox quickview tab: if you see something questionable, move curser to that area over the data dot. Go back to raw data and scroll over to the right. Look for drug name and concentration. Now look at the raw data at the problem area.
7. to change the scale, click on the axis > right click > format axis > scale > set scale.
8. Codes:
    1. green diamond   DMSO only
    2. blue square   Valinomycin
    3. orange circle   controls

9. On format tab: control C, copy redox filename (eg. 20100304r-AM1r-p0001a)
10. File > save as > jumble/copy/Cyan data open run date folder
11. Control V (paste name) and add underscore FINAL in the filename box.
12. Also change the "r" or "j" to "b" for **b**oth dyes
13. (eg. 20100304b-AM1b-p0001a_FINAL)

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Verified by:_____Date:___6/1/2012_____   _____

                        Print Name   Sign Name