

Hedrick FACS guidelines (Becton Dickinson FACSCalibur)

Jenny DuRose – 09-25-06

- Turn on FACS machine (button is on the right side of machine near back)
- Turn on computer.
- Before getting started, check the water in the tube and see if particles are in there, replace with clean water if needed. Run water through on high to clean out machine. If machine will not run, check the pressure on the reservoir tank.

Connect to network and mount disk:

APPLE

CHOOSE

Select apple share and find computer and mount drive.

Open cellquest application:

APPLE

CELLQUEST

Open template:

FILE

OPEN template file; see below for how to make a new template.

ACQUIRE

CONNECT TO CYTOMETER

ACQUIRE

ACQUISITION & STORAGE

Collection criteria: set number of events in G1=R1 before stopping

Storage gate: ALL

ACQUIRE

COUNTERS

(expand counters and select acquire to see how many events occur in gate)

ACQUIRE

PARAMETER DESCRIPTION

- press FOLDER button to select folder to save data to
- press FILE NAME button to name files

CYTOMETER

INSTRUMENT SETTINGS

- press OPEN button to find settings file
- press SET button to set those settings
- DONE

You will need to adjust the settings if you are working with different combinations of stains or different cell types. See Instrument settings section below.

RUNNING YOUR SAMPLES:

Run water through FACS on high (press HIGH button, and RUN button on FACS) and check to see that there are no cells in machine.

With the setup box checked, press the ACQUIRE button, and you should see no events. To stop press PAUSE.

Place your sample in machine, and press RESTART to clear the data. Check the flow rate, find appropriate flow rate using LOW, MED, and HIGH buttons on FACS machine. Modify the gate if needed to include the population of live cells. When cells look good, press PAUSE, then unclick the setup box, and press RESTART button.

You are now collecting the data. Keep an eye on how many events in your gate are collected, when that number is satisfactory, press PAUSE, and SAVE. If you do not press SAVE you can either RESUME, or RESTART. Restarting will erase the data collected.

Replace tube with your next sample, press RESTART to acquire your next sample. Do not press RESUME or else your new data will include the previous samples data.

CLEAN UP:

1. Run 5% bleach through machine on HIGH for a couple of minutes.
2. Run water on HIGH for couple of minutes.
Press ACQUIRE again to see that the machine is clean.
3. Set FACS to LOW and STANDBY.
4. Open reservoir drawer and flick depressurize switch.
5. Remove waste tub, dump in sink.
6. Remove reservoir and fill up with FACS buffer. You have to take off plate first.
7. Replace waste tub and FACS buffer, put plate back on and repressurize.

If someone is signed up to use the machine next, leave everything on.

At the end of the day, shut down the computer first and then power down the FACS machine.

INSTRUMENT SETTINGS:

You should bring tubes with unstained and single stained cells to work out instrument settings.

1. CYTOMETER DETECTORS/AMPS

With unstained cells modify the gate to fit live cells, and work out voltages for all channels using 4-color template. Unstained cells should be in center of SSC & FSC, and low in all the color channels. Use dropdown menu to set voltages for cells size -@ top for smallest cells, -@bottom for big cells.

2. CYTOMETER COMPENSATION

FL-1: FITC/GFP FL-2: PE FL-3: PerCP/PECy7 FL-4: APC/Cy5

Use single stained cells, compensate so that each dye is high for the correct channel, and low for the incorrect channel. You can only compensate for adjacent channels, so if the dye is really bright you need to compensate first for furthest away channel, and then the adjacent channel. (Example: if FL-1 is bleeding into FL-3, first remove FL-2 from FL-3, and then remove FL-2 from FL1)

The compensation window will show:

FL-1 -  %FL-2

This means that you subtract FL-2 from FL-1. So if FL-1 is high, increase the % so you subtract more FL-2. Also use the quadrant statistics to make sure that the cells aren't too squished down on the bottom of the axis. In this case you would decrease the amount of FL-2 subtracted from FL-1.

Compensate for all channels with the individually stained cells, and possibly secondary antibody controls if needed.

Save your settings:

CYTOMETER
INSTRUMENT SETTINGS

In menu press SAVE, and change the file name, and save to the appropriate folder.

Now you are ready to run your samples. YAY! 😊

MAKING A NEW TEMPLATE:

Open Cellquest:

APPLE
CELLQUEST

Open a new file:

FILE
NEW

If needed, you can adjust the number of pages you template is on:

FILE
DOCUMENT SIZE you can add or subtract pages.

WINDOW

SHOW PAGE BREAK uncheck the page break, its annoying.

Add FSC SSC dot plot:

PLOTS
DOT PLOT
Plot Source: set to ACQUISITION
X: FCH-H
Y: SSC-H
NO GATE (you want to see all events here)
Show: 1000 DOTS (this prevents the window from getting too cluttered with all the events.)

On your scatter plot you can gate for live cells, now you can make plots for the different stains, here is one example.

PLOTS
DOT PLOT
Plot Source: set to ACQUISITION
X: FL-2 (CD8-PE)
Y: FL-4 (CD4-APC)
Gate: G1=R1 (you want to see only live cells here)
Show: ALL (you will see all events within the gate accumulated)
HISTOGRAM you can set the x-axis similar to above and compare to number of events

FL-1: FITC/GFP
FL-2: PE
FL-3: PerCP/PEcy7
FL-4: APC/cy5

You can add statistics, for example:

Click on the plot that you want stats for.

STATS

QUADRANT STATS (gives you % in each of the four quadrants)

REGION STATS (gives you stats for within a gate)

To delete a gate:

GATE

REGIONS LIST

Click on the gate to be deleted, and press DELETE on keyboard.