COLUMBIA UNIVERSITY
FACS Facility User Guide

Location: 701 W. 168th St, Twelfth Floor, Rm 1211A
Hours: 24/7 (LSRFortessa) Cell sorting 10-5 (by appointment)

Lab Manager: Amir Figueroa (212) 342-4089 af2615@columbia.edu

Sample Preparation and Acquisition:

Tubes: Falcon polystyrene tubes (12X75mm, Cat#2008, non-sterile; #2052 (without cap), #2054 (with cap), and #2058, sterile.

Acquisition on LSRFortessa Cytometer-Approx. 2 x 10^e7 cells per mL (range: 0.5-2 x 10^7) resuspended in PBS containing 1% BSA. No phenol red should be used. Minimum sample volume is 500 uL.

Cells: If the primary cell type or cell line you are working with has a tendancy to form aggregates, you should treat the sample with an anti-agglutinating agent (see next paragraph) and then filter each sample prior to sorting through filter top tubes (Falcon12X75mm Cat#2235). These sterile tubes have a 35 micron mesh filter in the cap.

For primary cells and cell lines that have a propensity to form cell aggregates, there are a number of reagents that can be employed to reduce or eliminate the formation of aggregates. Pluronic F-68 sold by Sigma (Cat #P5556, 100mL sterile 10% solution) is a non-ionic, non-toxic surfactant ideal for cell sorting and sample acquisition. Dilute into the sample to give a final concentration of 1% vol/vol. Other reagents are: DNAse, EDTA (1 mM) or PBS w/o Ca++ or Mg++ containing 0.1% BSA or FCS.

Other commercially available products are AccuTase for confluent cell lines and AccuMax for Adherent cell lines. Both products are available through Innovative Cell Technologies/Phoenix Flow Systems http://www.innovativecelltech.com/

Rates: Rates are available on the CU Department of Microbiology & Immunology website under “Shared Equip/ Core Facilities” and listed under CU Department of Microbiology & Immunology “Flow Cytometry Facility”.

Instruments:

BD LSRFortessa. This instrument is a multi-parameter cell analyzer. It is operated by users after basic training in operation, maintenance, and troubleshooting. It runs BD FACS DiVa software. The LSRFortessa has four lasers with excitation lines at 407nm, 488nm, 561, and 640nm. It can collect up to 13 fluorescent parameters and two light-scatter parameters.

Basic Instrument Setup and Use of the LSRFortessa analyzer:
1. Your PI must be listed on the instrument Login and you must know your lab's password. If your PI is not listed, you must notify the facility lab manager with your account number for billing and complete basic operator training to obtain a Login and password.

2. Before starting up or using the instrument, make sure that the sheath system is turned on. If this is not engaged air will get into the system.

3. Start the computer if not already on and Login to the Windows OS. Next, click the desktop icon for the FACS DiVa software. If the instrument is already on, the Log-in window for DiVa will already appear on the screen. Select your PI and enter the password for your lab.

4. The system will display a message it is connecting or connected to the cytometer. If it has not connected, go to CYTOMETER pull-down menu and choose "Connect"

5. Under the folder of your PI will be the individual User folders. Open your folder and create a new experiment or use a previous experiment as a template and choose "Duplicate without data"
6. Click once on the first tube in the list to be acquired. Click the turquoise-colored tab "Parameters" and delete those fluorochromes not being used. Check the box for FSC-H (Height) in addition to the defaults of FSC-A and SSC-A (area). The addition of FSC-H can be used for a doublet discrimination gate in conjunction with the FSC-A parameter.

7. If you are running a multi-color experiment, select "Experiment" from the top menu line and from the resulting pull-down menu, choose "Compensation Setup" > "Create Compensation Controls.

8. Under Compensation Controls in the Browser List, select "Unstained" and place the unstained sample on the cytometer. Adjust the sensitivity for FSC and SSC to give a good separation between cells and the noise and debris occurring in the lower left corner of the dot display. Use the adjustments to place the unstained peaks in the first decade of the histogram of each fluorochrome present. The Gaussian-shaped peak should be centered on the 10^e2 tick mark of the four decade Log scale.

9. Acquire each compensation control. When finished acquiring all single-colored stained compensation control tubes, select “Experiment” > “Compensation Setup” > “Calculate Compensation“.

10. Click on arrow to left of the first sample tube in the Browser List. To observe and verify calculated compensation values will be applied to acquired samples, click the turquoise tab “Compensation”. Values should be displayed in the right column for the fluorochrome pairs being compensated. Verify the check box “Apply compensation” is checked. The voltages are no longer changed after this step or the compensation will be invalid.

11. If you wish to enter specific info about the experiment, click “Specimen” and enter the source material. Clicking on the generic tube_xxx, allows you to give the specimen a specific name.

12. Before acquiring the first sample, check for noise and debris in the lower left section of your dot display. The default threshold trigger parameter is FSC at channel 5,000. Adjust higher if excessive debris and noise is present. You can also add a secondary parameter. In most cases, click “Add” and SSC parameter will be added. Make sure to check the radio button for “AND” instead of “OR” to and start at channel 5,000. Certain applications such as DNA measurement for cell cycle require FL-3 (PI channel) as the threshold trigger.

13. On the Acquisition Dashboard, Stopping Gate, Storage Gate, and Events to record parameters can be changed from the default values. The “Stopping Gate” defines the gate in which the number of events are to be acquired while the “Events to Record” can be set for a specific number of events to be acquired in the file. The defaults are “All Events” and 10,000. Frequently the “Stopping Gate” will use one of the gates that defines a population to guarantee a minimum number of the target population is collected.

14. Press RUN button on instrument front panel and acquire your sample by clicking “Acquire Data” In this mode, specimen events will be displayed in real-time. When ready to collect (save) the data to the disk, click “Record”
The sample will be saved automatically when it reaches the stop event count. If your sample runs out before enough events are collected, Press “Stop Acquisition” to save all the events collected to that point. All instrument settings and compensation are saved with the data file and can be recalled for future experiments using the same sample type.

15. Run your samples, changing the Sample ID for each sample.

16. Once all your samples are acquired place a tube of 10% bleach on the SIP and run on high flow rate for 5 minutes. Follow this with a tube of DI water for another 5 minutes before placing in STANDBY.

17. Log out of DiVa software to end the billing period

Data, Acquisition Template and Instrument Settings storage

Data may be analyzed by FACS DiVa or FlowJo. If your lab needs a copy of FlowJo, please contact the facility manager for more information on how to obtain.

Each user is responsible for backing up his/her data files. Data can be transferred via LAN to your lab server. Data can also be transferred to a flash memory drive. Data files are left on the LSRFortessa FACS Station hard drive for 30 days. After one month, the data is removed.

Data should NOT be stored on the instrument computers. Templates and Instrument Settings may be stored within your personal folder in the DiVa Browser hierarchy. To ensure safekeeping of your templates, create a folder marked “Templates” and make copies for acquisition by using the “Duplicate without Data” sub-menu.