

COLUMBIA UNIVERSITY

Department of Microbiology & Immunology

FACS Facility FACS Aria Sorting Guide

Location: 701 W. 168th St, Twelfth Floor, Rm 1211A

Hours: 24/7 (LSRII)

Cell sorting: 10-5 (by appointment)

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

Sample Preparation and Sorting:

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

Sorting on FACS Aria II:

Sample Tubes: Falcon polystyrene tubes (12X75mm, Cat#2054 or 2058, sterile with cap) or Falcon 15mL conical (Cat#2096).

Collection Tubes: Same as above except for 4-way sorting tubes must be 12X75mm. Collection tubes should contain an appropriate volume of media supplemented with 20% FCS. No phenol red should be used.

Cell Concentration: Approx. 2×10^7 to 3×10^7 cells per mL resuspended in PBS containing 1% BSA. No phenol red should be used. Minimum sample volume is 500 μ L. For single cell sorting into 96 well plates concentration should be $1-5 \times 10^6$ cells per mL.

Cells: Samples should be filtered prior to sorting through filter top tubes (Falcon 12X75mm 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”.

Instrumentation:

BD FACSAria II

This instrument is a high-speed cell sorter capable of separating the cells for subsequent assays or culture. The Core Facility lab manager operates it. Like the LSR II, it runs FACS DiVa software. It has five excitation lines at 355, 407, 488nm, 561, and 640nm. It can collect up to 14 fluorescent parameters. It can sort 1-4 separate populations simultaneously, or perform single cell sorting into 6,12,28,48 and 96 well plates as well as microscope slides.

A dedicated manager operates the FACS Aria II with input from the researcher on creation of the acquisition display configuration, gating logic and sort gates for collection of target cell population.

Data, Acquisition Template and Instrument Settings storage

Data may be analyzed using 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 files are left on the LSR II FACS Station hard drive for 30 days. After one month, the data is transferred to an external hard drive for an additional period of storage

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.