University of California, Los Angeles

Broad Stem Cell Research Center

BSCRC - Flow Cytometry Core Facility User Policy

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A. GENERAL CONDITIONS:

1. Use of the Flow Cytometry Core (FCC) Facility (TLSB and Factor) is restricted to members of the Broad Stem Cell Research Center (BSCRC).

2. Each lab member utilizing the core will need an agreement signed by both the user and their PI acknowledging the privileges and responsibilities prior to receiving access to the FCC Facility. If a user changes labs, a new agreement form signed by the new PI is required.

3. The instruments in the Core are shared equipment and all investigators must have equal opportunity to run their experiments. Please make sure you follow the basic rules of using shared equipment:
   a. Make appointments only when you need them and show up on time.
   b. Cancel/reschedule in advance so that others can use the time.
   c. Fill the tank/empty waste as instructed.
   d. Shut down when you are the last user.
   e. Back-up data and remove/delete your experiments from the DIVA browser.

4. Failure to follow the Core policies may result in loss of access to the Core.

5. The use of infectious agents such as yeast, bacteria, replication competent viruses, etc. is not allowed in the FCC Facility. The use of potentially infectious materials including those listed below requires consultation with the Core Director.
   a. Use of any human materials other than established cell lines requires consultation with the Core Director about potential biohazards – please note SOP for BSL2 samples.
   b. Cells transduced with viral vectors require consultation – please note SOP for BSL2 samples.
   c. Some experiments may need to be done at other Flow Cytometry facilities.

6. No one under the age of 16 is allowed in the BSCRC FCC Facility for any reason.

7. All scheduling and training requests are managed through PPMS (https://ppms.us/ucla/).

B. SERVICES OFFERED:

1. <u>SORTING (BD FACSARIA):</u>
   a. No training required. Only the BSCRC FCC staff may operate the cell sorters.

   b. Scheduling sorting is requested through https://ppms.us/ucla/, on a first come, first served basis. Sort appointments can be booked no more than 2 months in advance. Large sort appointments are subject to FCC director approval.

   c. Sorting fees are $102 per hour charged at the amount of time used. Users are required to provide the appropriate FAU, approved by their PI, when scheduling an appointment.

   d. Consultation and 1st trial run (per PI, not per user) will not be charged.

   e. Billing starts at the time of the booked reservation time, rounding to 15-minute increments and includes set-up time.

   f. There are three different nozzles available: 70um, 85um, 100um. The default setting is 100um nozzle. You must select a nozzle size at the time of scheduling the appointment. The table below

shows how many cells we can sort hourly for each nozzle:
Up to 14 um cells – 70 um – 60 million cells/hour
14-17 um cells – 85 um – 30 million cells/hour
17-20 um cells – 100 um – 20 million cells/hour
If you are unsure which nozzle is appropriate for your experiment, contact the FCC operators directly.

g. Cell collection options include: Eppendorf tubes, FACS tubes, 15 mL Falcon tubes, 6 well plates, 12 well plates, 24 well plates, 96 well plates, 384 well plates.

h. Up to four populations can be collected at once. Please note, up to two populations can be collected simultaneously into a 15 mL Falcon tube and only one population at a time can be sorted at once on a plate.

i. Cancellation Policy: Reservations must be canceled in PPMS. Cancelations with less than 24-hour notice will result in a charge of 50% of booked time. No shows (i.e. failure to cancel the reservation through PPMS) are billed for the full time of the reservation.

2. ASSISTED ANALYSIS (LSRII, FORTESSA, AND CELESTA)
   a. No training required. Assisted analysis will be conducted completely by the BSCRC FCC staff.

   b. Schedule assisted analysis directly with the FCC Operators by emailing bscrcflowcore@mendet.ucla.edu. Scheduling is dependent on instrument and FCC Operator availability. Consultation may be necessary.

   c. Assisted analysis fees are $102 per hour charged at the amount of time used. Users are required to provide the appropriate FAU, approved by their PI, when scheduling an appointment.

   d. Cancellation Policy: Reservations must be canceled via email to FCC Operators at least 12 hours prior to the scheduled appointment time. Cancellations made less than 12 hours in advance will result in a charge of 50% of booked time. No shows (i.e. failure to cancel via email) are billed for the full time of the reservation.

3. INDEPENDENT ANALYSIS (LSR II, FORTESSA, FACTOR LSRII, AND CELESTA):
   a. Training: Training requests are made through https://ppms.us/ucla/. Two types of training are required before investigators can independently use the analyzers: two web-based BD courses and two one-on-one hands-on training sessions with the core operator.

      i. Prerequisite web-based training:
         a) Intro to Flow Cytometry
         https://www.bdbiosciences.com/en-us/support/training/e-learning-courses

         When completing the online course, after answering Question #3 in the Data Analysis Section, please save an electronic version and upload it when you make the training request through PPMS.

         b) BD FACSDiva™ Software Basics Training
         https://www.bdbiosciences.com/en-us/support/training/e-learning-courses
         When completing the online course, save an electronic version of the DIVA Software Worksheet Quiz page and upload it when you make the training request through PPMS.

      ii. Request training through PPMS (https://ppms.us/ucla/).

      iii. Submit a Flow Cytometry Core Agreement signed by the user and the user's PI to BSCRCTLSBAccess@mednet.ucla.edu. Trainee must visit the BSCRC administrative office

in 477 BSRB to gain ID card access to the FACS Core facility, Monday or Thursday between 3:30-4:30PM.

iv. Hands on training with an FCC operator:
   a) An FCC operator will contact you to schedule two one-on-one hands on training sessions once they receive confirmation of receipt of access agreement. Hands on trainings take place Monday and Tuesday from 1-4pm. If a user is unavailable during this time, the FCC operators will try to accommodate a different time, but it will be dependent on instrument and operator's availability.
   b) First time users will be given an individual software account and password access, discuss data management, learn how to run the first experiment and learn how to perform instrument maintenance (start-up, shut down).
   c) Users are required to bring the following to their training appointment: unstained cells, single color controls and a fully stained sample. For example for a 3 color experiment you will need unstained cells, FITC stained cells, PE stained cells, APC stained cells, and FITC+PE+APC stained cells.

   d) Please email the FCC operators (bscrcflowcore@mednet.ucla.edu) the fluorophores that you are going to use. Users will need about one million cells per tube.

v. Successful completion of all parts of the training is required for independent use of analyzers, and will likely require more than the minimum time. Certification will be granted at the discretion of the FCC operator overseeing the training. Large gaps in analyzer usage may result in the need for re- training.

vi. Special experiments may require usage of instruments after normal operating hours. After-hours operation requires an additional hands-on training session at no charge to the user. After-hours (7pm -9am) is subject to approval by the FCC Core staff. Schedule training directly with the FCC operators to review turn on/off of instruments, filling sheath tank, empty waste and running QC beads on the instruments. Submit completed forms to the Terasaki building manager and BSCRC admin staff to gain access to the building and FCC facility after- hours. PIs are required to provide an expiration date for after-hours access. Use of the LSRII analyzer on the 14th floor of the Factor Building is available to all authorized analyzer users during normal business hours. Outside normal business hours, access to that machine is restricted to users who already have access to the Factor Building.

vii. Because the Celesta is equipped with a high throughput system 96 well plate reader, additional training is required at no charge to the user. Contact the FCC operators directly to schedule an appointment. Training is subject to instrument and operator availability.

viii. For training fees please see section C.

b. Scheduling: Scheduling time on the analyzers is done through the PPMS website: https://ppms.us/ucla/

   i. Each user is required to log onto the machine using her/his own personal PPMS user ID and password. The user scheduled on the calendar is the person who should be using the instrument.

   ii. Independent analysis is charged at the amount reserved or the amount of time used, whichever is greater. Independent analysis is $36.51 per hour.

   iii. Cleaning the analyzer after samples are completed is mandatory for use of the equipment. **Failure to clean the equipment will result in loss of access to the Core.** During the requested timeslot, you are required to include the extra time necessary to clean the analyzer

(15 minutes) and the transferring of data from the database to your personal external drive or Cloud System.

iv. Failure to follow instructions provided (leave instrument on, run unattended - except for HTS, fill sheath tank, empty waste container, leave instrument ON overnight, not performing cleaning procedures, not removing/deleting your experiments from the DIVA browser, etc.) results in an instrument misuse fee. See section C- Pricing below.

c. Cancellation Policy: Reservations must be canceled in PPMS at least 12 hours prior to the scheduled appointment time. Cancellations made less than 12 hours in advance will result in a charge of 50% of booked time. No shows (i.e. failure to cancel through PPMS) are billed for the full time of the reservation.

4. CONSULTATIONS:
   a. Consultations with a Core Operator are available during the weekly drop-in office hours on Mondays 10:30am to noon, TLSB 3129.

C. PRICING:

1. FEE SCHEDULE:

	Name of Product/Service	Rate	Rate Basis
1	Independent Analysis	$36.51	per hour
2	Assisted Analysis	$102.00	per hour
3	Assisted Sorting	$102.00	per hour
4	Training - initial session	$102.00	per session
5	Supplemental training	$50.00	per hour
6	Instrument misuse fee	$102.00	per event

D. CANCELATION POLICIES:

1. INDEPENDENT ANALYSIS: Reservations must be canceled in PPMS at least 12 hours prior to the scheduled appointment time. Cancelations made less than 12 hours before the scheduled appointment time will be billed for 50% of the booked analysis time.

2. ASSISTED ANALYSIS: Reservations must be canceled in PPMS at least 12 hours prior to the scheduled appointment time. Cancelations made less than 12 hours before the scheduled appointment time will be billed for 50% of the booked assisted analysis time.

3. SORTING: Cancellation Policy: Reservations must be canceled in PPMS. Cancelations with less than 24-hour notice will result in a charge of 50% of the booked sorting time.

4. NO SHOW POLICY*:* No shows (i.e. failure to cancel in PPMS) are billed for the full time of the reservation.

E. RECOMMENDATIONS/GOOD PRACTICES:

1. Cell suspensions must be passed through a 35 µm nylon mesh filter (BD catalog # 352235) right before sorting/analysis. Other filters available include, Partec CellTrics (10um, 20 um, 30um or 50 um) and BD Falcon Cell Strainers (40um, Falcon catalog # 352340).

2. Clearly label all samples indicating the contents. This includes the accurate labeling of collection tubes and extra media.

3. Users must always bring compensation samples. Preferable concentration of the controls is 100,000 cells/mL, volume 0.5-1ml. If you do not have enough cells for compensation, you may purchase compensation beads from:

a. BD Biosciences cat # 552845 for mouse samples and cat #552843 for almost all human samples.
b. ThermoFisher– UltraComp beads cat # 01-222-42

4. Basic suggested recipe for sorting buffer:
   a. 1x PBS (Ca2+/Mg2+ free)
   b. 1 mM EDTA
   c. 25 nM HEPES (pH 7.0)
   d. 1% Fetal Bovine Serum (heat inactivated)
   e. Sterilize through 0.2µm filter, store at 4C.

5. Re-suspending cells in Ca^{2+}/Mg^{2+} free media containing EDTA (up to 5mM) during sample preparation can help prevent the formation of clumps. If cell clumping is due to released DNA, addition to DNase II at 10 U/µL may help. However, please check that this will not adversely affect your sample or the labeling regime used.

6. The sample concentration should be 5-10 million /ml. Please bring additional buffer in case is necessary to dilute your samples.

7. We recommend sacrificing a few sorted cells at the end of the sort to do a purity check.

8. Collection tubes:
   a. In order to prevent cells sticking to the side of the tubes, pre-coat the tubes, filling them with serum or media for 30 min before the sort.
   b. Up to 1/5 of the collection tube should be filled with collection media (1-3 mL for 15 mL collection tubes, 0.5-1 mL for 5 ml collection tubes, 100 µL for 96 well plates and Eppendorf tubes).
   c. Antibiotics are recommended for media to be sorted into. Please bring extra media and collection tubes.

F. SPECIAL REQUESTS:

1. Some dyes/fluorochromes require physical replacement of the filters in the instrument.

2. Only the FCC Operator or the approved designee may make changes to the filters. <u>24 hour advanced notice is required for such changes.</u>

3. Reservations must include an additional 30 minute lead time prior to starting the experiment.

4. Sorting into 384 plates will require an extra 30 minute set-up time.

G. ACKNOWLEDGEMENTS:

1. All publications and presentations that include use of the core should acknowledge support from the "Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research University of California, Los Angeles Flow Cytometry Core Resource."