

All new Flow Cytometry Core users must take our Instrument Operation Course prior to gaining access to iLabs, and our Introduction to Flow Cytometry class when it is offered (monthly).

Scheduling

- i. Analyzers are available 24/7 for trained users, and can be reserved through iLabs.
- ii. Reservations are voided if the user does not arrive within 30 minutes of the start time. After 30 minutes, the instrument is available to any user.
- iii. You must STOP so the next user may start their appointment on time, and begin cleaning procedures 10 minutes before your appointment time is over so that you are finished on time.
- iv. Users will be charged for whichever is greater of time scheduled versus actual time used.
- v. Cancellation policy: Users must cancel their reservations 24 hours in advance of the scheduled appointment to avoid paying for the reservation time. If a cancellation occurs <24 hours in advance, iLabs calendar will allow for other users to schedule your unused time. In this circumstance, you will only be charged for time that it isn't utilized by another user.

Safety

- i. Users must always wear gloves when using the analyzers and associated computers.
- ii. No eating, drinking, food or beverages are allowed in the laboratory area at any time.
- iii. Users analyzing human, non-human primate or potentially infectious materials must fix their samples. Users who need to run the above materials unfixed must contact Flow Core staff during the planning stages of the experiment so that accommodations can be made to have analysis performed on our BSL2+ Influx Sorter.

Operation

- i. All samples must be filtered prior to analysis with 40-70 micron mesh.
- ii. Before each run, users must check the level of the waste tank and empty/add 300mL of 100% bleach to the bottom of the tank as needed. User must also refill the sheath reservoir so that it is full at the start of the run.
- iii. Performance checks are run on the morning of each scheduled calendar day by Flow Core personnel. It is strongly recommended that each user perform their own QC using beads (Core provided) prior to each run.

- iv. All users must run and record shut-down procedure (10% Bleach 1 minute with the arm over, followed by 5 minutes on HIGH. Repeat with distilled water).
- v. Data acquired on Flow Core equipment must contain the proper identifying information for parameters used, including the fluorescent dye and molecule/marker/antigen/protein.
- vi. Users should export their data to the server immediately after their run. Data files left on the hard disk for more than a week may be deleted.
- vii. Internet use is not permitted on any of the Workstation computers.
- viii. After hours users are to shut off the instrument at the end of their session, unless the next user is physically present to take on responsibility of the instrument.
- ix. To report problems with any of the flow cytometers, please contact Dave Dunaway at dave.dunaway@nationwidechildrens.org
- x. Failure to adhere to Flow Core operational policies will result in a \$100 charge for each occurrence. The first 3 occurrences are free (email/reminder). Situations that accrue charges:
 - 1) Running the sheath dry
 - 2) Leaving the instrument on overnight
 - 3) Improperly prepared samples that result in a clogged instrument.
 - 4) Alteration of software/configuration or machine without authorization
 - 5) Failure to run/record proper shutdown procedure
 - 6) Recording of data with generic placeholders in place of experiment-specific labels.

I. Sorter Rules

Scheduling and Safety

- i. All sorts require the completion and approval of a **Sorter Sample Questionnaire** and a **Sort Protocol Questionnaire**. It is the user's responsibility to inform the Flow Core about cellular source material *and* to have appropriate IBCSC approvals.
- ii. If this is your first sort of the month, place a request for sorting in iLabs. This must be placed, and attached to a cost center, *prior* to any sorting appointments for the month.
- iii. Please contact Dave Dunaway for approval/scheduling. To facilitate scheduling, please refer to the read-only sorter schedule posted in iLabs to request a specific morning or afternoon reservation.
- iv. Please be punctual to your scheduled sort time with properly prepared samples. Charges start at your reservation time.

- v. Users will be charged for whichever is greater- time scheduled, or actual time used.
- vi. Users who cancel the sort <48 hours in advance will be charged for their entire scheduled time, unless the time gets used by someone else.
- vii. We charge \$66/hour for sorting plus 60 minutes for setup/shutdown procedures.
- viii. The sorter is available M-F 10am-5:30pm. After hours sorting may be available upon request, at a rate of \$100/ hour.

Operation

- ix. All samples must be filtered prior to sorting with 40-70 micron mesh
- x. To prevent clumping please consider:
 - a. Keeping the concentration of fetal calf serum or BSA <0.5%
 - b. Use of PBS without Ca⁺⁺/Mg⁺⁺
 - c. anti-clumping agents such as EDTA
 - d. Preferred sample concentration 0.5-1 x10⁷/mL for smaller cells (PBMCs/lymphocytes) and 1-2 x 10⁶ for larger cells (fibroblasts, hepatocytes, etc.).
- xi. Unstained cells and single-color controls must be provided for each sort, even if the initial setup is expected to be the same.
- xii. To preserve the integrity of your sample, collection media should contain added serum. If many cells are expected, up to 100% serum can be used for collection, to be diluted with sheath as the cells are sorted. This is highly cell-type/experiment specific.

II. RESPONSIBILITIES

- i. All users must pay for use of Flow Cytometry Core services – no development work for free
- ii. Users are responsible for exporting raw data files to the server after the end of every acquisition session. The Flow Cytometry Core deletes all data from the analyzer workstations every 2-4 weeks. The Flow Cytometry Core agrees to maintain a data archive of all raw data files and these files are available upon request.
- iii. For any Service (ELISPOT, Nanosight, acquisition, consulting, sorting, etc.), all users must have a request submitted in iLabs with the appropriate cost center number prior to appointment time.
- iv. Flow Cytometry Core will hold regular user oversight committees for feedback
- v. Flow Cytometry Core will make available instrument performance and calibration records.