Sorting at the Salk's Flow Core Version 1.3

❖ **Download this instrument Help File:** salk.edu/fccfaccess > Help Files

Before coming to the core: Planning a FACS experiment

Core email: FCCF@salk.edu

- **Check scheduling availability:** Submit a Sort Request form through Infinity (click submit samples icon > fill out *Sort Request Form *COVID-19 Update**), and if you're new to sorting (or it's a new project) contact FCCF@salk.edu to check you have every thing in order (e.g. check that your fluors that can be used, enough sorter time available, what controls to bring). Keep in mind that most sorts at Salk (adherent cells) use a 100um nozzle, allowing us to run about 10million cells per hour through the sorter. This is just a guideline: throughput is reduced if samples are sticky or have a lot of debris. A pilot experiment is recommended to more realistically assess sort duration for your prep (and inform sample prep optimization!).
- **Fluor selection, collection options:** If purchasing reagents, we can help select colors that will give you the most flexibility across the 4 sorters. Occasionally, microscopy application fluors are brought to the core that we cannot excite effectively. Instrument configurations can be viewed on the intranet in our Help Files section. The sorters can sort up to 4- (Fusion) or 6- (Influxes) populations *simultaneously* (for bulk sorting into tubes), and can accommodate a variety of tube types and multi-well plates.
- **Understanding how to prepare a sort ready, high quality sample:** For cell/tissue derived samples, a DNAse I treatment step should be included (there are many places it can be added into your workflow: for details download the DNAse I info sheet from salk.edu/fccfaccess > Help Files). DNA mediated viscosity contributes to sample stream "fuzzing" (impacts purity) and reduces sort speed. It impinges on precious sorter time because frequent stops will be needed to dry off the high voltage plates and run bleach through the sample line (to remove the DNA coating the sample line and nozzle). For samples that have a lot of debris, use of gradient clean up steps (percoll, sucrose and iodixanol cushions etc. for different types of cell and organelle preps) can also be helpful for obtaining high quality preps needed for successful sorting. Samples should be freshly filtered through a strainer/mesh before running on the sorter to minimize the chance of a nozzle clog (potentially up to 30min lost if nozzle clogs).
- **Cell concentration, Sort Buffer and Collection Buffers:** Concentration ranges for sort sample ranges from 8-15million/ml for non-adherent cells (e.g. lymphocytes) and up to 8 million/ml for adherent cells (depending on the cell type; e.g. neurons: 1-3 million/ml for better efficiency sorting).

  *For sterile sorting applications: Be sure to include Antibiotic/Antimycotic (commonly Pen/Strep or anti/anti) in the Sort and Collection media as "insurance" through out the sorting process. Bring extra Sort buffer to the appointment so samples can be diluted down for optimal sort efficiency at the sorter.

  Sort buffer: Ideally is a low viscosity (< 5% protein) pH buffered solution designed to keep cells viable for the duration of the process. Ideally it is colorless (e.g. no phenol red/low optical background), but this may not be an issue unless working with extremely dim fluorescence. Typical base compositions are PBS, HBSS (for more sensitive cells), or a specialized cell media with up to 5% serum (protein additives should be kept low to minimize sample viscosity). pH buffering is an important component (as pH will drop due to the sample being pressurization), usually in the form of HEPES.

Component	Purpose	Notes
Ca/Mg free PBS or HBSS, specialized cell media (ideally phenol-red free)	Cell viability/sample stability	PBS is common, HBSS or media more suited if viability is an issue for delicate cells.
Suitable protein additive, other relevant protein/small molecule additives for cell type - (less than 5% total protein)	Cell viability/sample stability	FBS or BSA are common. LIF/ROCK inhibitors are examples of stem cell molecules which may be considered. Try reducing if fanning is an issue (test to see how sample viability/stability is affected): Fanning can be determined in a pilot sort or test sort streams with sample running.
25mM HEPES, pH7	pH buffering	Protect sample from low pH while under pressure
Optional: 2.5mM EDTA,	Inhibit cell:cell adhesion	May not be necessary for all cell types (can determine in pilot sort)
Optional: antimicrobials	For sterile applications	Pen/Strep or anti/anti commonly used
Optional: DNAse I	Maintainence dose for long durational treatment over the timeframe of the sorting.	May not be needed if DNAse clean step done earlier and sort is not overly long. Utility can vary depending on sample and sort needs. It is most effective to include a standalone clean up step (refer to our DNAse Help File for more details) before bringing to sorter. Free DNA is a leading cause of stream fanning issues at the cell sorter.

Collection buffer: we can collect into whatever is needed for your downstream assay; keep in mind that this will be diluted by PBS from the sorter. Contact us for volume estimates for each nozzle (posted in the core at the sorters: on Fusion hood, and Influx-1).

- **Viability dyes:** Recommended for samples that may have dead or dying cells, or where high viability is important (including RNA extraction). These come in many colors and are either DNA labeling (enters plasma membrane compromised cells) or amine labeling (labels plasma membrane compromised apoptotic cells brighter due to easy access to the intracellular amines). These are sometimes combined with Annexin-V conjugated in a similar color to enhance exclusion of apoptotic cells. Unless the channel is already taken (e.g. using BFP, CFP or other 405nm excited fluors), DAPI (a DNA dye) or Zombie-Violet (amine reactive dye) are the two easiest to use because they suit most common fluor combinations with minimal fluorescence overlap.
- **Get Ready:** Obtain your reagents (DNAse I, any viability dye etc.) and plastic-ware (the correct tubes, cell strainers)!

Day of the sort appointment: Checklist of what to bring with you

- Lab coat
- Single cell suspensions (filtered for clumps using a strainer) in tubes ready to run. For long duration sorts: you can leave some samples in whatever you spun down in e.g. 15ml falcon, and will we filter into FACS tubes before running. Filtering too far in advance doesn't help as cells will have time to re-form clumps.
  - To run, samples have to be in Falcon brand 5ml polypropylene FACS tubes – *catalog no. 1495911A* (buy sterile tubes with snap on cap)
  - Make sure your samples are properly secured: For sterility, cover the blue strainer caps – *catalog no. NC1827548* with parafilm if needed, or replace with snap on plastic cap before transporting. LCMV and patient samples must be closed with a snap on cap.
- Collection vessels with media ready to sort into: you may sort into Tubes or Plates. Please talk with FCCF staff prior to sort appointment in order to determine what the best option is for your experiment.
- Extra tubes (including for collection) and extra cell strainers (for multiple samples or longer sorts, we can filter prior to running). Prepare samples in a staggered fashion for best viability for long sorts/multiple samples.
- Extra sort and collection buffers
- Information regarding your experiment either via email or written out on paper:
  - Your fluorescent panel, including any live/dead stain, fluorescent proteins, and any potential auto fluorescent channels you need to include.
  - Gating strategy you would like to use.
  - How many cells you need sorted out per sample (approximation).
  - BSL safety level information.
- **The core has:** gloves, single wrapped sterile transfer pipettes (squeeze type), packets of tubes available for recharge. We *usually* have some sterile pipette tips in different sizes, but please bring your own if you plan on doing a lot of precise pipetting of reagents requiring tips (especially at the Fusion which is a shared user instrument, if you don't trust the shared pipette tips!).