

NEW USER TRAINING**CELL SORTING GUIDELINES**

PROCEDURE

1.0 Cell Sorting at the ACSF

- 1.1. Schedule an individual meeting with the Advanced Cytometry & Sorting Facility (ACSF) staff to discuss the design of your specific sort. Consider the following:
 - A. **Sample Type and Size:** Species, tissue, blood, bone marrow, cell lines, tumor, etc.
 - B. **Number of Cells Needed:** Determine the number of sorted cells you need to collect for downstream experiments. Calculate the number of cells you need to start with based on the sort population frequency and assuming 70% recovery of cells of interest.
 - C. **Single Cells and Enrichment:** Ensure single cell preparation via homogenization, digestion, RBC lysis, washing, etc. Consider enrichment with magnetic beads or other steps.
 - D. **Reagents:** Design your panel using FluoroFinder or other panel design tools to choose antibodies and viability dye. Plan an antibody titration experiment on an analyzer, if needed.
 - E. **Cell Sorter Selection:** Choose a sorter considering nozzle size, pressure, temperature, sample agitation, collection device, collection media, and fluorochrome detection capabilities.
 - F. **Single Cell Suspension:** The cell concentration should be 10-20 $\times 10^6$ cells/mL in PBS or media with 0.5% or less FBS or BSA. Aggregation can be prevented by adding 2 mM EDTA. All samples must be filtered through 40 μ m filters prior to sorting. Avoid creating bubbles.
 - G. **Controls:** Include single stained beads or cells for compensation, positive and negative biological controls, and gating controls such as FMOs.
 - H. **Safety:** Inform ACSF staff of any potentially infectious agents present in your sample.
- 1.2. Schedule adequate time on the sorter for experiment set up, sorting, purity checks, and training. Sorters can process a maximum of 30-60 $\times 10^6$ cells/hour based on sample type and quality.
- 1.3. During sample sorting on the instrument remember to:
 - A. Double check the gating strategy so that sort populations are mutually exclusive.
 - B. Monitor the sample rate and stream image throughout the sort.
 - C. Perform sort purity checks, if needed, and save your experiment template for future use.
- 1.4. Analyze data on any of the available post acquisition software platforms such as FlowJo, Kaluza, or FCS Express. Schedule time with the ACSF staff for training on the software.

RELATED PROCEDURES

This handout is related to ACSF SOP TR001. Please see the full SOP for further information.