OBJECTIVE/PURPOSE:
Requirement for Sorting Experiment at UNC Flow Cytometry Core

RATIONALE:
To provide clear information about required controls needed for Cell Sorting Experiment

EQUIPMENT:
1. Prepared solution to be sorted, stained appropriately, and filtered in appropriate tube (12x75mm (5mL) polystyrene or polypropylene plastic tubes)
2. Beads for compensation (if required); Ultracomp eBeads™ Plus Compensation Beads preferred (ThermoFisher, cat 01-3333-41)
3. Sorting Controls, as defined by staff
4. Collection Tube(s): 12x75mm (5mL) polypropylene plastic tubes or 12x120mm (15mL) polypropylene.
5. Additional collection media

PROCEDURE:
1. Before the sorting experiment has been scheduled, the Client must meet with a member of the Flow Core Staff to discuss the experiment and the required controls needed for that experiment.

2. Client will be required to bring to the Sorting Experiment Compensation Controls for each source of fluorescence present in the cells. Compensation controls are defined as either a) antibody capture beads (we recommend ThermoFisher Ultra Comp beads), b) cells stained singly with each antibody to be used in the experiment, or c) highly expressed fluorescent protein. The single stained samples must have a clear positive. Compensation controls must either have a native negative population in the control tube or a unstained sample matching the cell type autofluorescence must be provided.

3. The Client may also be required to use a live/dead discriminator dye in the sorted sample and bring a single-color stained control for that dye as well.

4. The Flow Core will require that the sorted sample or a subset of that sample to be evaluated post sort by the operator and the machine to determine the purity of the sorted sample where that is practical to perform.

General Recommendations for a Successful Sort

Please review the 'Practical Cell Sorting' document prior to your Sorting Experiment. We stress the importance of being knowledgeable of your cells, the Core will provide generalized recommendations, but it will be important you optimize these suggestions to your cell type and experiment. Important points are highlighted below:

1. Bring sample(s) in a clean, single cell solution. Debris should be kept at a minimum as these can affect purity.

2. Optimal cell concentration varies by cell type and conditions. Generally, adherent cells should be brought at a concentration of 10 million cells per mL; suspension cells at a concentration of 30 million cells per mL. If there are less than 10 million cells in either case, bring in 500 uL of buffer media. In all cases, bring additional buffer media to dilute the sample if necessary.

    a. If single cells are being collected in a plate, bring all samples at a concentration of 1 million cells per mL.

3. The buffer cells are brought in can aid or hinder cell viability through the sorting process. Generally, we recommend Hank's Balanced Salt Solution (HBSS) or a culture medium (e.g. RPMI-1640; if possible the media should not have phenol red) the cells will thrive in. Include 2% BSA or FBS. Bring additional buffer media in case staff needs to dilute samples.

4. Bring cells on ice

5. Collection tubes should be polypropylene when possible. The tubes should contain a collection media in the tube when brought to the core. The media should be buffered with a non-CO2 based buffer (e.g. HEPES) to maintain pH. The media should contain serum or BSA at an initially high concentration (e.g. 10 - 50%) that will get diluted (with the droplets from the sorter, sterile PBS) as the tube fills with sorted cells.

    a. For the 12x75mm (5 mL) tubes place ~1ml of media in the tube.
    b. For the 12x120mm (15mL) tubes place ~2ml of media in the tube.
    c. If collecting single cells in a plate, use ~200 uL of conditioned media.
    d. When using the 70µm tip we can collect ~3×10^6 cells per 5 mL tube.
    e. When using the 100µm tip we can place about ~1×10^6 cells per 5 mL tube.
    f. When collecting into the 12x120mm (15 mL) tubes increase the numbers above by 3-fold.

6. If sorting adherent cells, gentle dissociation is key. We recommend Accutase.

7. Adding DNase at a concentration of 0.1 mg/mL will get rid of free-floating DNA that can cause your cells to clump together. The use of Mg^{2+} free and Ca^{2+} free media as well as the addition of EDTA (1-5mM) will also reduce clumping of the final cell suspension.

8. If growing cells after sorting, include a strong antibiotic in the collection media as well as the culture media for a few days post sort. We highly recommend Gentamicin or Ciprofloxacin as the antibiotics. Note, we are thorough in our cleaning of the instruments daily and between clients, but we cannot guarantee the room is sterile. We insist any sample preparation and/or staining be done in the client's lab under aseptic conditions. As with anything, the more a sample is handed around and fiddled with, the higher the chance of contamination from the environment.
9. BSL 2 Transporting Samples: https://www.med.unc.edu/flowcytometry/rates-services-policies/biosafety/#:~:text=Transporting%20Samples%20to%20the%20Flow%20Facility

10. UNC Flow Cytometry Core Facility, General Lab Rules and Collection Tubes: https://www.med.unc.edu/flowcytometry/instrumentation/general-lab-rules/

REFERENCES:

1. ThermFisher UltraComp eBeads: https://www.thermofisher.com/order/catalog/product/01-3333-41#/01-3333-41
2. Practical Cell Sorting at UNC Flow Cytometry Core Facility: https://www.med.unc.edu/flowcytometry/wp-content/uploads/sites/744/2019/04/Practical-Cell-Sorting-2019-2.pdf

REVISIONS:

SOP version Number	**Date**	**Tracked Changes** (clearly list changes made and why)	**Employee**
LPP SOP10.2	10/25/2023	Added website link on transporting samples to and from Flow Core	Ayrianna Woody