

## Cityof Analytical Cytometry Hope Core

## **Cell Sorting Guidelines**

- 1. The optimum sorting concentration
  - a. for the MoFlo and the 100-micron nozzle tip is no more than 8 million total cells/ml.
  - b. for the Aria sorters:
    - i. 70 micron tip: between 8-10million cells/ml
    - ii. 85 micron tip: no more than 5million cells/ml
- 2. Sorting samples should be resuspended in media with low serum (1-2%), 1X PBS, or other buffer cells prefer.
- 3. The final pellet of the controls and the sort sample(s) need to be resuspended in the same buffer.
- 4. For the controls, we need at least 200,000 cells in 500ul final volume.
- 5. Negative controls can be unstained cells, isotype controls, secondary reagent only, or FMO depending on your application.
- 6. For multicolor staining, please provide single color compensation controls (no viability dyes) and a completely unstained aliquot of your sorting cells.
- 7. For sterile sorts, all samples (controls and sorting samples) need to be treated in a sterile fashion.
- 8. For 1 or 2 way bulk sorting, sorting can be performed into 15ml conical centrifuge tubes with 2-3mls collection media. Also, 12X75mm culture tubes (with 1-2ml collection media) or 1.5ml microfuge tubes (with 200-300ul collection media) can be used.
- 9. For 3 or 4 way bulk sorting, we need to sort into either 12X75mm culture tube or 1.5ml microfuge tubes (same volumes as in #7) with 1-2mls
- 10. Collection media can be complete media or media with 20% serum.
- 11. For plate sorting, place appropriate complete media in each well.
- 12. Appointments should be scheduled at least 1 week in advance. Some weeks are busier than others, so we cannot guarantee that a slot will be available.
- 13. Documentation of noncompetency needs to accompany any samples that are transduced with lentiviral vectors. The sort will not be performed if the documentation is not provided.