

## Pre-Sort Sample Requirements (Updated Jan 2024)

Please note: the CCG will NOT sort infectious samples. Our system is not housed in a Biosafety Hood.

1. All samples **must** be resuspended in BD FACS Pre-sort buffer (Cat #563503). Aliquots, 5ml, are available for purchase at the CCG. Yeast and bacteria samples are the only exceptions and must be resuspended in the Sheath buffer: 1X PBS.
2. All samples **must** be filtered to remove clumping prior to sorting through a 35um-40um mesh cell strainer, (Corning 431750). CCG has 40um cell strainers available for purchase. Yeast sample should be sonicated and filtered prior to sort.
3. Sample volume should be ~1.5ml with a cell density of  $5 \times 10^6$  to  $1 \times 10^7$  cells/ml. The minimum sort volume is 200ul but from experience we recommend at least 1ml.
4. Adherent cells **must** be released from surface with Accutase. The CCG has 5ml aliquots of Accutase for purchase (BD Accutase Cat # 561527).
5. Please bring samples in 5ml Falcon plastic capped tubes (Falcon #352054) and collection tubes or plates of your choice (types listed below). Also, bring any media you want your samples to be sorted into collection tubes, otherwise samples will be collected in sheath buffer (1X PBS). In general, you will only need around 5ml of media since usually 0.5 ml is add to collection tubes.
6. Indicate the fluorochromes you wish to use for your sort. If you have more than 1 fluorochrome to sort in your sample then you need to bring addition controls, 1 for each fluorochrome.
  - Example of 2-way sort with cells expressing GFP and mCherry
  - Controls Required:
    - (a) Unlabeled control
    - (b) GFP only expressing control
    - (c) mCherry only expressing control
7. Indicate the size cell type and ~ size of cells to be sorted (reference below).  
Bacteria (0.5um), Yeast (4um), Red blood cell (6um), Lymphocyte (8um), Monocyte (14um)
8. Indicate if you want an Aseptic sort (charged for additional cleaning time, ~90min) or Normal sort. We prefer to do Aseptic sorts on Wednesdays in case there are issues with autoclaving buffers and supplies. Yeast and bacteria sorts will be charged for additional cleaning time required after the sort.
9. Indicate the Sort Mode you wish, please note that single cell mode requires a microplate to sort into.
  - a. Purity: If you need a highly purified sample. In this case, the droplet containing 2 cells of interest will be collected. If one of those cells is not of interest the droplet will not be collected. Lower efficiency and generally takes longer to sort sample.
  - b. Yield: If you need to obtain the maximum yield for your target population and do not need a pure sample. In this case, the droplet containing 2 cells will be sorted

if 1 of them is a cell of interest. Lower purity but very high efficiency and shorter run times.

- c. Single Cell: If you need an exact cell count. If you need to sort single cells into plate wells. In this case, the machine will only take droplets with 1 cell in the drop. Please note, you can sort more than a single cell into each well of your plates, an index sort. For example, you might want 10 cells per well that are your cell of interest. The system can do up to 100 cells per well under this setting.
10. Please indicate the sort temperature you wish to have your samples collected in: 4C, 22C, 37C or 42C.

Collection tube options:

1.5ml, 2.0ml Eppendorf tubes

5.0ml Falcon tubes (same as the ones you bring your sample in)

6 to 384 well culture plates (Index sort)

Microscope slide (Index sort)

The system is calibrated every day the machine is prepped for a run. Your run specs will be email to you after the sort. You may request the daily calibration file as well.