Sample Preparation for Cell Sorting

If you have not performed cell sorting with our facility, please consult Core staff prior to your sort. To request a consultation or reserve appointments, navigate to the Versiti iLab website: https://versiti.ilab.agilent.com

Biohazards and Sample Handling Safety:

- When working with biohazardous samples (i.e., derived from human sources, containing potentially infectious material, containing recombinant DNA), disclosure of sample information to Core staff via submission of a "Biosafety & Sample Disclosure" form is required for all sample types before a sorting experiment can be performed. This form helps the core determine which biosafety level and instrument setup is necessary for your sort. The core will refuse any sample that has not been cleared prior to sorting.
- If you are sorting the same sample on a repeated basis, only one form is needed for that cell type UNLESS THE CELLS HAVE BEEN MODIFIED OR TREATED IN ANY WAY. You must submit an updated form to sort cells derived from the same tissue type/culture line if the sample has been treated with an infectious agent, viral vector, drug, plasmid, etc., that has not been previously communicated to Core staff.

Sample Preparation Recommendations for Cell Sorting:

- ALL samples coming into contact with the sorter MUST be prepared under aseptic conditions with clean, filtered reagents. THIS INCLUDES SINGLE STAIN CONTROLS FOR COMPENSATION.
- Cells should be re-suspended in medium containing buffer (recommended: PBS or HANKS Ca/Mg^{++} free) which protects cells from pH changes occurring during sorting. See additional recommendations below:
    - 10-25mM HEPES buffer (additional buffering capacity)
    - 1-5% FBS or 0.1-1% BSA (promotes single cell suspensions and overall cell health)
    - EDTA, 2- 5mM concentration (may help prevent cation-dependent cell-cell adhesion)
    - Phenol red-containing media CANNOT be used
- For fragile samples or when cell viability starts off at a low level, omit EDTA and add 25-50 µg/mL DNase with 5 mM $MgCl_2$. (This digests free DNA released by dead cells which can negatively impact suspensions.)
- Samples should be prepared in polystyrene (PS) tubes. The Aria cell sorter can accommodate 5-mL round-bottom tubes, 15-mL conical tubes, and bullet tubes. (5-mL tubes are preferred.)
- Re-suspend samples at 5-10 million cells per mL, or the recommended concentration as discussed during sorting consultation. The minimum sample volume should be ~500 µl.
    - For rare populations or large cells: it is recommended to re-suspend samples at 2-5 million cells per mL. The minimum sample volume should be ~500 µl.
    - **For single cell sorting into 96-well plates, the cell concentration should be 1.5 million per ml.**
- **Cells MUST be filtered just prior to sorting with a 30 µm cell strainer. We recommend Sysmex CellTrics 30 µm sterile filters (#04-004-2326).**
- Target cells can be collected into the following formats: 5-mL round-bottom tubes, 15-mL conical tubes, snap-cap/Eppendorf tubes, 96-well plates (U-, V-, and flat-bottom), other plate styles, and slides.
    - **Collection tubes/plates must contain medium for cells to be collected into.** When sorting cells for subsequent culture, we recommend that the media you place in the tube or well be buffered with a non-CO_2-based buffer (e.g., HEPES). If not buffered, media will become very alkaline and kill cells.
    - Sample and collection tubes must be STERILE.
    - **Polypropylene (PP)** is recommended for collection.
- Bring samples for sorting on ice unless your cell type is not stable at 4 °C. The starting sample and sort collection can be temperature regulated upon request**.**
- Bring the appropriate controls for your experiment. This may include one or more of the following:
    - Unstained control (a wild type, untreated sample) and isotype control (optional)
    - Secondary-only control (if you are using a primary + secondary probe)
    - Single color-stained controls for each fluorophore in your panel (AKA: compensation controls). For compensation controls, please stain for a high copy number antigen on your cells OR use compensation beads (e.g., CD4-FITC, CD4-PE, CD4-APC for lymphocyte single stains).
    - Positive control, if available (representative of the most unique outcome)
    - A viability marker (e.g., DAPI, 7-AAD, PI) to exclude low quality, dead cells