Preparing cells for sorting

- *Please use appointmentquest to sign up for cell sorting*

Sample disclosure

- **A sample disclosure form is required for all NEW sample types before a sort will be scheduled. This form helps the core determine which biosafety level is necessary for your sort, and what set-up is required for your cell type. 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. (e.g. you may sort the same strain of animal tissue, or cultured cells but a new form would be required if the sample had been treated with an infectious agent, viral vector, drug, plasmid, etc)**

Sample preparation

- ALL samples coming into contact with the sorter MUST be prepared under sterile conditions with sterile reagents. THIS INCLUDES SINGLE STAIN CONTROLS FOR COMPENSATION. This reduces the risk of contamination.
- Cells should be re-suspended in medium containing buffer. (buffer in the media protects cells from pH changes occurring during sorting, serum will promote single cell suspension)
    - Ex) 25mM HEPES buffer, 4-5% FBS or BSA and PBS or HANKS.
    - You can also add EDTA at up to 5mM concentration. It may help to prevent cation-dependent cell-cell adhesion.
    - Also add 25-50ug/ml of DNAse I and 5mM $MgCl_2$ to the buffer. It may help if cells are clumping due to the cell death.
- Resuspend cells to at least 10 million per mL. **Cells MUST be filtered just prior to sorting with a 30 um cell strainer. We recommend Sysmex CellTrics 30 um sterile filters, #04-004-2326**
- Collection tubes must contain medium to be collected into. This can be similar to sorting media.
- Sample and collection tubes must be STERILE. Polypropylene is recommended, as polystyrene will collect charge and cells will stick to the tubes and dry out (die) certain tube sizes and profiles do not fit. The following tube types are recommended
    - For 15 mL tubes
        - Falcon, # 352097
        - Corning # 430790
    - For 5 mL tubes
        - Falcon # 352063
    - For micro tubes
        - 1.5 mL eppendorf
    - For plates**: please bring an example plate for the core to test prior to plate sorts**
- Bring cells for sorting on ice, unless your cell type is not stable at 4C. The collection block 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)
    - Isotype control
    - Secondary only (if you are using a primary+ secondary probe)
    - Single stain for each color for colors that have spectral overlap
        - For compensation controls, please stain for a high copy number antigen on your cells, OR use compensation beads (ex, use CD4-FITC, CD4-PE, CD4-APC for lymphocyte single stains)
    - Positive control if available

If you are new to flow cytometry and have questions regarding panel design or gating strategies, please consult the core staff prior to your sort. This will make your sort more efficient and reduce on-demand troubleshooting. Please contact Core Manager for details.