CHILDREN'S MEDICAL CENTER
RESEARCH INSTITUTE
AT UT SOUTHWESTERN

SAMPLE PREPARATION FOR SORTING

For quick and efficient sorting, please follow the facility guidelines below.

1. **Suspend viable cells in a low protein buffer.**

    - High protein concentrations can disrupt sort stream formation. We typically use two percent FBS in Ca/Mg-free PBS with 0.5 mM EDTA, although any variation of this will generally work (BSA, RPMI, HBSS, etc.). Protein additions and media should be sufficient to keep the cells alive for the duration of the sort.

    - Sorting adherent cells adds a level of complexity to an experiment. The cells must be disassociated to pass through the sorter, and this is often done with trypsin. The quickest and most common neutralization method is to add FBS to the cells. It will neutralize the trypsin effectively but will also reintroduce the components that caused the cells to adhere. Using a soybean trypsin inhibitor will mitigate the re-adherence.

    - It is highly recommended to use a viability dye in your staining panel. This will help eliminate dead cells.

2. **Filter cells through nylon mesh (70 microns maximum) immediately prior to sorting. This will prevent a nozzle clog, which could inadvertently stop the stream or disrupt cell acquisition.**

    - If your cells are susceptible to clumping (such as with adherent cells), sorting the cells in Ca/Mg-free buffers, as well as adding 0.5 mM EDTA and DNAse (25-50 µg/ml), may reduce aggregate formation. This will minimize the chance of a nozzle clog, which will impede sort performance.

3. **The FACSAria II SORP, FACSAria Fusion SORP, and FACSymphony S6 can accommodate 15 ml conical tubes and 12 x 75 mm Falcon (Becton Dickinson) polystyrene tubes for your sample.**

    - The optimal sort speed is typically ¼ the droplet generation frequency; therefore, an overconcentrated sample will reduce purity on the back end. Bring some dilution buffer to add to an overconcentrated sample.

    - Cell concentration should be no more than $30x10^6$ per ml for a sorting session.

4. **Collect sorted cells into tubes or plates.**

    - Collection tubes may be 1.5mL Eppendorf tubes, 12 x 75 mm round bottom tubes, or 15 ml conical tubes.

- During a sort, cells travel in a buffered saline. This is not optimal for keeping cells alive over a long period of time. You can improve cell recovery by adding buffer with protein into your collection tubes. Typically, 10 to 50 percent protein in this buffer is sufficient.

- To further improve recovery, incubate your collection tubes with a buffer solution containing protein. This will neutralize the plastic charge, reducing cell adherence to the tube wall. Alternatively, using polypropylene tubes, which are less charged than polystyrene, will help provide the same outcome. The FBS also provides a "cushion" for the cells when they "land" at the bottom of the collection tube. Prepare enough of these tubes ahead of time and deliver them with the cells to be sorted.

- If you're sorting into media, ensure the media is HEPES buffered. Buffers such as RPMI are formulated to buffer in a CO_2 atmosphere (like the atmosphere found in your lab's incubator) and, as such, don't buffer well in our normal atmosphere.

- Collection plates can be between 6 and 384 wells.

5. Temperature control

- The FACSAria II SORP, FACSAria Fusion SORP, and FACSymphony S6 are equipped with a temperature-controlled sample chamber and collection tube holder to keep both pre- and post-sorted cells between 4°C and 42°C.