CHILDREN'S MEDICAL CENTER
RESEARCH INSTITUTE
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SAMPLE PREPARATION FOR SORTING

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

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

   High protein concentrations can disrupt sort stream formation. We typically use 2 percent FBS in Ca/Mg-free PBS with 0.5 mM EDTA, though any variation on this (BSA, RPMI, HBSS) will generally work. 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 have to 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. Be careful of this - though it neutralizes the trypsin effectively, it also adds back all the components that cells need to readhere to each other. Try to use soybean trypsin inhibitor instead.

   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 to prevent nozzle clogs.**

   If your cells are particularly susceptible to clumping (as are many adherent cells), sorting the cells in Ca/Mg-free buffers, adding 0.5 mM EDTA and DNAse (25-50 µg/ml) may reduce aggregate formation, which can clog the sort nozzle and impede sort performance.

3. **The FACSAria II SORP/FACSAria Fusion SORP can accommodate 1 ml microtubes and 15 ml conical tubes in addition to the 12 x 75 mm Falcon (Becton-Dickinson) *polystyrene* tubes.**

   Know the cell count at the time the cells are going onto the sorter – NOT from when you first began preparing them.

   Because an optimal sort speed is typically ¼ the droplet generation frequency, overconcentrating the cells will reduce purity at the back end. Bring some dilution buffer with you just in case the cells are too concentrated.

Cell concentration should be no more than $30x10^6$ per ml for the FACSAria II SORP/FACSAria Fusion SORP.

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

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

   Cells are going travel in a buffered saline. This is not conducive for keeping cells alive for long periods of time. You can improve recovery by ensuring that the catch buffer has some – but not too much – protein in it. Typically, only 10-50 percent protein in the catch buffer is sufficient.

   To improve recovery, precoat (incubate your plastic tubes with a buffer solution containing protein) the tube with protein or buffer to neutralize the plastic charge. Even better, use tubes that are polypropylene, which is less charged than polystyrene, thus reducing “droplet spray” and tight adherence of the sorted cells to the walls of the tube. The FBS also reduces cell adherence to the walls of the tube and provides a "cushion" for the cells when they "land." Prepare a sufficient number 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 CO2 atmosphere (like the atmosphere found in your lab’s incubator) and, as such, don’t buffer well in our normal atmosphere.

   Plates can be between 6 to 384 wells plate.

5. **Temperature control.**

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