Cell Sorting Tips

Cell sorting can be a scary proposition. A precious sample is introduced into an instrument that pressurizes the cells, moves them past one or more lasers, then vibrates the stream at high frequency before decelerating the cells to atmospheric pressure before they hit an aqueous surface. It's a difficult journey. Here are ten things that can help achieve a successful sort:

1. **Always count the cells.** Know the cell count at the time the cells are going in the sorter – not from when the cells were first prepared. Since an optimal sort speed is typically one-quarter the droplet generation frequency, overconcentrating the cells will reduce purity at the back end. Bring some dilution buffer just in case the cells are too concentrated.
2. **Use a soybean trypsin inhibitor.** Sorting adherent cells adds a level of complexity to an experiment. The cells have to be disassociated and this is often done with trypsin. The quickest and most common neutralization method is to add fetal bovine serum to the cells. While this neutralizes the trypsin effectively, it also adds back all the components that cells need to re-adhere to each other. Try a soybean trypsin inhibitor instead.
3. **Design proper antibody panels.** When trying to define a cell population, include both positive and negative markers in the antibody panel. The use of dump channels, negative markers, and multiple positive markers will help ensure that the sorted cells are what they are supposed to be.
4. **Use a viability dye.** Make sure to include a viability dye in the staining panel to help eliminate dead cells.
5. **Filter the cells.** Nothing is worse than a clogged nozzle when sorting. It adds time to the sort and reduces efficiency. Just before sorting, pass the cells through an appropriate-sized filter to remove cell aggregates. You can look at cells under a microscope to ensure that there are no clumps prior to sorting.
6. **Pre-coat the collection tubes**. Incubate plastic tubes with a buffer containing protein. This will help reduce/eliminate the charge on the plastic. Since the droplet containing the cells is charged, it can be attracted to the charge on the plastic. This results in the droplet hitting the side of the tube wall, and the cell dying as the small volume of liquid evaporates. To prevent this, precoat the tube with protein/buffer to neutralize the plastic charge. Polypropylene is less problematic than polystyrene.
7. **Use appropriate collection buffer.** Cells are going to be traveling in a buffered saline solution. This is not conductive for keeping cells alive for long periods of time. Improve recovery by ensuring that the collection buffer has some – but not too much – protein in it. Typically 10-50% protein is sufficient.
8. **Add HEPES buffer.** If you're sorting into media, make sure the media is HEPES buffered. Buffers like RPMI are formulated to buffer in a CO2 atmosphere (like that found in an incubator) and don't buffer well in a normal atmosphere.
9. **Keep cells cold (or warm).** Cells respond to temperature differences. Some cells do not like to be kept cold and will die quickly if sorted in a 4^{o}C buffer. Sometimes an intermediate temperature such as 15^{o}C can be advantageous.
10. **Be aware of the cell population's threshold settings.** The higher the threshold, the easier it is to visualize the specific cell population. But this doesn't eliminate the possibility that debris and junk are still present within the cell population. With a high threshold t the sorter is ignoring it. Here's the key: whatever the cell sorter ignores will end up in the final sorted population. For example, people have sent "pure" populations of sorted mature B cells for sequencing only to learn that hemoglobin (from contaminating red blood cells) is the most abundantly expressed gene in their sample.

Adapted from Biorad "Advanced Cell Sorting Tips"