Here are notes about how to prepare your samples for cell sorting:

1. Cell concentration (before sort) should be about 2 – 5 million/mL. All samples must be filtered (minimum 40 micron pore) before sorting.

2. **Bring extra pre-sort buffer.** Buffer in which to bring your cells, although some variation is acceptable:

a. *Optimal* - BD Biosciences Pre-sort buffer (cat#563503)

b. 1 x Phosphate Buffered Saline (PBS) +1% Fetal Calf Serum (Heat inactivated) or 1% Albumin (for sticky cells)

c. Hanks Balanced Salt Solution (HBSS) (Ca2/ Mg2 Free) 25 mM HEPES pH7.0

1% Fetal Calf Serum (Heat inactivated) or 1% Albumin (for sticky cells)

3. For samples with a high dead cell concentration or primary tissue preps: Dead cells can release their DNA into sorting media which in turn can cause cells to clump together.

Strategies to reduce cell clumping

a. BD Biosciences pre-sort buffer

b. Include 50% Accumax (Invitrogen) in sort buffer

c. Treat cells for 15-30 minutes in a sterile solution of 100 ug/mL DNAse I and 5 mM $MgCl_2$ in HBSS at room temp. Wash the cells 1x in HBSS containing 5mM $MgCl_2$.Resuspend the cells in HBSS containing 25-50 ug/mL DNAse I, plus at least 1mM $MgCl_2$ prior to and during the sort. (5mM $MgCl_2$ is optimal)

4. For bulk collection, **bring media with a high FBS content (20-50%)** to help cells recover from the sorting process.

a. For direct plate collection, bring plates loaded with the culture media appropriate for the sorted cells.

b. For post sort mRNA analysis, cells can be sorted directly into RNA isolation reagents, such as Trizol-LS, RTL buffer, RNA Later, etc. Please ask for more details.