

Cell Viability, autofluorescence and cell aggregation may all affect the quality of cell sorting experiments. Good sample preparation is crucial and will result in better sort purity, yield and post-sort cell function and viability.

### 1. Buffer Suggestions

- Use  $\text{Ca}^{++}/\text{Mg}^{++}$  free buffers → reduces cell aggregation
- Use BSA (0.1 – 1%) or dialyzed FBS (1 – 5%)
  - Minimal amount of BSA decreases autofluorescence, increases population resolution, and improves flow rate in instrument
  - Avoid non-dialyzed FBS as it facilitates cell-cell adhesion by replacing Ca and Mg
- Add EDTA (2 – 5 mM) → helps prevent cell adhesion
- Add 10 – 25 mM of HEPES to improve pH stability
- Add DNase I (25 – 50 ug/mL) and 5 mM of  $\text{MgCl}_2$  → digests free DNA released by dead cells.

### 2. Single Cell Suspension

- Filter samples immediately before sorting
- Avoid keeping cells at unnecessarily high concentration. Keep cell suspension at 1 – 10 million/mL during processing.
- Always check cells under the microscope as you are preparing them to ensure they are in a single cell suspension.

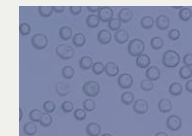
### 3. Dead Cell Discrimination

- Strongly recommended to use a Dead Cell Exclusion Dye with any cell sorting experiment. It will greatly reduce autofluorescence and lower non-specific baselines, which will result in increased population resolution.

#### To keep in mind:

- ✦ Use minimal speed to centrifuge the cells.  
*A good starting point is 300 g for 10 minutes.*
- ✦ Avoid vigorous vortexing.
- ✦ Do not generate a dry pellet at any time during processing.
- ✦ Avoid introducing air bubbles, since surface tension forces can kill cells.
- ✦ Keep cells on ice, unless otherwise required by a specific protocol.

Good Sample  
Preparation



Poor Sample  
Preparation

