**General Guidelines for Flow Cytometry**

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Sample Prep</th>
<th>Optimal Buffer**</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC (from frozen) OR Any sample w/ high cell death</td>
<td>DNase to prevent clumping: Per sample – 1mL RPMI (w/ 10% FCS) + 5µL 1M MgCl₂ + 10µL DNase I aliquot</td>
<td>FACS Wash Buffer (1% BSA): 1L bottle DPBS (w/o Ca^{2+} &amp; Mg^{2+}) + 2mL 0.5M EDTA + 10g of BSA</td>
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<tr>
<td>Most Other Samples</td>
<td>Varies. Depends on cell type.</td>
<td>Same as above but can vary.</td>
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<tr>
<td>Cell line (adherent)</td>
<td>0.25% trypsin</td>
<td>Sorting buffer w/ 1mM EDTA</td>
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<tr>
<td>Compensation Tubes (beads or cells)</td>
<td>EXACT same fluorochrome and conditions as your samples (fixation, incubation times/temps, etc)</td>
<td>Same as your sample</td>
</tr>
</tbody>
</table>

**If staining with more than 1 Brilliant fluorochrome, you must add Brilliant Stain Buffer before making your master mix to prevent cross-reactions/staining artifacts in your sample data.**
When preparing samples for flow cytometry there are many factors that can impact results, such as centrifugation speed, temperature, and buffers. Determining the buffer composition for a certain sample type is an essential part of protocol optimization. The choice of protein source and additives as well as their correct concentration is crucial to improve the sample quality, especially when sorting cells.

**BUFFER**
- PBS (phosphate buffer saline) or HBSS (Hank’s balanced salt solution)
- **Do not use Phenol Red**. It may impact the ability to detect fluorescence.
- **Avoid buffers with Mg²⁺ and Ca²⁺**. The presence of Mg²⁺ and Ca²⁺ can increase cell clumping. Exception: when using Annexin V, for apoptosis studies, make sure the buffer contains Ca²⁺.

**PROTEIN**
- FBS (fetal bovine serum) or BSA (bovine serum albumin)
  - It adds some vital components making cells viable for a longer period.
  - Prevents non-specific binding so cells will be less sticky.
  - High concentrations impact the stability of the sorting stream and can increase autofluorescence.
- FBS final concentration should be between 1-2%. As a blocking agent, it can be used at 10%.
- BSA final concentration should not be higher than 0.5%.

**ADDITIVES**
- HEPES and/or EDTA or DNase
  - **HEPES** increases the CO₂ buffering capacity, stabilizing the buffer pH for a longer time. Very important to keep cells alive during long periods out of the more optimal conditions, like cell sorting.
  - **EDTA** prevents cell clumping. The ideal concentration is from 0.5 to 5 mM.
  - **DNase** should be used when a lot of cell death is expected. The concentration should be between 25-100 µg/mL. It requires MgCl₂ (1-5 mM) as a co-factor. Do not use with EDTA.

Buffers can be house made or purchased from several commercially available options. Keep in mind that there are other special buffers needed in flow cytometry, such as the fixation and permeabilization buffers or buffers that stabilize the use of polymer dyes (see Flow Post it).