General Guidelines for Flow Cytometry

	Analytical cytometer	Sorting: 100um nozzle	Sorting: 70um nozzle
Sample Concentration	1-20 x 10^6	5-10 x 10^6	30-50 x 10^6
Acquisition Rate	Acq. rates vary, running in medium speed is recommended	No more than 8,000 events/sec	No more than 22,000 events/sec
Filter Requirement*	35um FRESHLY filtered. Need to refilter after 2 hours. *Gut, Brain, and Skin: Filtering is a must!		
Collection tubes	N/A	Media w/ HEPES and antibiotics	
Fixation Requirement	All HIV, Covid-19, and any infectious samples should be fixed; fixation inactivates infectious agents that may be contained in the sample.		

Tissue Type	Sample Prep	Optimal Buffer**
PBMC (from frozen) <u>OR</u> Any sample w/ high cell death	DNase to prevent clumping: Per sample – **1mL** RPMI (w/ 10% FCS) + **5µL** 1M $MgCl_2$ + **10µL** DNase I aliquot	FACS Wash Buffer (1% BSA): **1L bottle** DPBS (w/o Ca^{2+} & Mg^{2+}) + **2mL** 0.5M EDTA + **10g** of BSA
Most Other Samples	Varies. Depends on cell type.	Same as above but can vary.
Cell line (adherent)	0.25% trypsin	Sorting buffer w/ 1mM EDTA
Compensation Tubes (beads or cells)	EXACT same fluorochrome and conditions as your samples (fixation, incubation times/temps, etc)	Same as your sample

****If staining with <u>more than 1 Brilliant fluorochrome</u>, you must add Brilliant Stain Buffer before making your master mix to prevent cross-reactions/staining artifacts in your sample data.**

Flow Post-its
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It's all about the buffer

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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** (1). It may impact the ability to detect fluorescence.
- **Avoid buffers with Mg^{2+} and Ca^{2+}.** The presence of **Mg^{2+} and Ca^{2+}** can increase cell clumping (2). Exception: when using Annexin V, for apoptosis studies, make sure the buffer contains Ca^{2+}. (3)

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 (4)
- **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 CO_2 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 $MgCl_2$ (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)