Cell Sorting Buffer Guide

Quick-reference guide for preparing biological samples for analysis or sorting

Purpose

This guide summarizes commonly used buffer components for flow cytometry analysis and cell sorting. Components are modular: not every reagent is necessary for every assay. Final buffer selection is subject to change depending on each sample type, assay design and staining strategy, fluorochromes, instrument and sort conditions, and downstream use. Feel free to contact the Flow Core at flowcytometry@cores.utah.edu with questions or for discussing specific scenarios.

Recommended Basic Buffer

Component	Final concentration / notes
1× PBS	Ca^{2+}/Mg^{2+}-free
EDTA	1 mM
HEPES	25 mM, pH 7.0
Fetal bovine serum (FBS)	1%, heat-inactivated
Sterilization / storage	0.2 µm filter; store at 4 °C prior to use

Breakdown of reagents and recommended uses

Component	Typical concentration	Primary role	When it helps	Important cautions / impact on outcome
PBS (Ca^{2+}/Mg^{2+}-free) Sigma, D8537	Base buffer	Optically clear isotonic buffer that helps maintain pH, osmolarity, and ion balance.	Most scenarios, standard starting point for analysis and sorting buffers.	Reduces cation-dependent cell–cell adhesion.
FBS or BSA	FBS/BSA: 5–20 mg/mL (0.5%–2%)	Binds to cell surface proteins blocking nonspecific interactions with antibodies.	Useful when samples are sticky.	Reduces cell-surface adhesion but reintroduces the cations that mediate cell adhesion.
EDTA ThermoFisher, 15576028	0.29–1.46 mg/mL (1–5 mM)	Chelates divalent cations required for many adhesion molecules.	Reducing cell clumping and improving single-cell suspensions.	It can improve dissociation but may be deleterious for some cell types or assays.
HEPES Sigma, H4034	2.38–5.96 mg/mL (10–25 mM)	Provides CO_2-independent pH buffering.	Especially useful on sorters that expose samples to high pressure or extended handling outside a CO2 incubator.	Improves pH stability while processing and during long sorts.
Sodium azide	5–10 ng/mL (0.05%–0.1%; 76.9–153.8 µM)	Limits bacterial growth, reduces photobleaching, and capping/internalization of antibodies or antigens.	Useful for fixed or staining workflows where preserving live-cell function is not required.	Highly toxic, mutagenic, and potentially explosive. Do not use it if cells must remain functional after sorting.
DNase I Sigma, D-4513	25–50 µg/mL	Degrades extracellular DNA fragments that promote aggregation.	Helpful in cell death assays, persistently clumping samples or	Usually employed alongside $MgCl_2$.

			containing tissue dissociation debris.	
$MgCl_2$	0.476 mg/mL (5 mM)	Provides Mg^{2+} needed for DNase activity.	Add when DNase is included.	Recommended concentration supports DNase activity without strongly promoting cell–cell adhesion.

Specific scenarios

Scenario	Recommended adjustment
Sticky cells	Increase EDTA to 5 mM and use FBS dialyzed against Ca^{2+}/Mg^{2+}-free PBS.
Adherent cells	Avoid quenching trypsin or detachment reagents with media or buffers that reintroduce Ca^{2+}/Mg^{2+} as these cations promote cell-cell or cell-surface adhesion. Use a cation-free FBS-containing buffer. Increase EDTA concentration but consider is the impact on your downstream assay.
Samples with many dead cells or tissue debris	Add DNase as needed to reduce DNA-mediated aggregation. Alternatively add 10 U/mL DNase II for dead/tissue-rich samples; verify compatibility with the workflow before use.

One note about filtering

Regardless of how much you tweak your buffer, a single clump of cells may still cause clogs in the nozzles or in the flow cell. These may disrupt the laminar flow, potentially stopping the sort or creating side-streams that decrease the purity of your collected cells. Both will require the sorter to cleaned and re-set which will cause some delays. Filtering your samples through a mesh—typically 30 to 70 microns—is very important to ensure a successful sort. We have some filter-cap tubes for our use, but we expect our clients to integrate this step into their sample preparation protocols.

- DNase and $MgCl_2$ should be mixed together and then added to the other buffer reagents.