

Cell Sorting Guidelines - Sorting Buffer & Collection Buffer

Critical: Cells must be in a single cell suspension throughout the sort

To that end, start with the Basic Sorting Buffer Recipe below and modify it according to the properties of your sample.

Basic Sorting Buffer:

- 1x PBS or 1x HBSS without Ca²⁺/Mg²⁺
 - 1 to 2.5mM EDTA
 - 25mM HEPES, pH 7.0
- 1-3% Heat-Inactivated FBS

Important: Filter samples through a cell strainer immediately before sorting. Keep cells on ice at all times

Sorting Buffer Modifications:

1. For Cells that easily Clump:

Increase the EDTA to 5mM. Use FBS that has been dialyzed against Ca/Mg.

2. For Adherent Cells:

Regular culture media or PBS/FBS solution will reintroduce the cations that support cell to cell attachment to each other, forming clumps “*de novo*” after trypsinization. Instead, quench the trypsin with buffer/media that contains dialyzed FBS. You may also consider additional, gentle pipetting up and down or increasing the EDTA concentration if the cell suspension still have clumps.

Accutase: Accutase is a more gentle alternative to Trypsin/EDTA for cell detachment. It better preserves surface antigens and doesn't need neutralization by serum containing buffers that would re-introduce divalent cations. Viability

remains high after even 30 minutes of Accutase exposure. Cell can remain in Accutase for sorting.

3. If your cell preparation generates a lot of dead cells:

A DNase I digestion [step] may improve the prep. Free floating DNA cause cells to clump. Importantly, DNase I activity is dependent on Mg^{2+} and Ca^{2+} , so you need to remove the EDTA and reintroduce the cations for the enzyme to work. Also, bovine pancreatic DNase I activity is highest at 37-40°C. Lowering the buffer temperature will render the enzyme less efficient. Consult with enzyme manufacturer for the best compromise between increasing the prep temperature/digestion timing versus potential loss of viability.

4. If you are sorting lymphocytes:

You can skip [or lower to 1mM] the EDTA on the sorting buffer, as they don't generally clump.

Collection vessels:

We can sort into 15mL Falcon, 5mL FACS, Eppendorf tubes or 96 well plates. Polypropylene is better than polystyrene; sorted drops contain a charge and will be attracted and stick to the charged polystyrene tube walls causing diminished recovery.

Collection Media

That obviously depend on the downstream application. Below are some general suggestions.

Important: As cells are sorted on a drop of sheath buffer, the collection medium is incrementally diluted as the sort progresses. Please check the "*Numbers to consider for Sorting*" table for details.

Single Cell Sorting for downstream cell culture:

Sorted single cells may struggle to survive. To increase the chances that a sorted single cell will survive post sort and form a colony, collect and grow the cells post sort on a 1:1 mix of conditioned media and fresh growth media initially.

Bulk Sorting for downstream RNA extraction:

Option 1: Use pure serum, media or PBS. Keep the cells in ice, spin them down to remove the buffer and then freeze or proceed immediately with RNA extraction using your protocol of choice

Option 2: Use RNA extraction buffer as collecting media. Vortex and snap freeze in dry ice. Keep in mind the RNA extraction buffer would have been diluted with sheath fluid and its efficacy will be diminished.

Bulk Sorting for downstream cell culture

You want the sorted cells to be collected onto the media that best preserve their viability. Still, that collection media will become diluted as sheath fluid is added with every sorted drop. Having an increased percentage of serum in the starting collection media is often recommended.