Scripps Research

Sort Buffer

The proper design of sort buffer for both your pre-sort sample and your collected sample is crucial for a successful sort. The following will be a basic recipe and some suggestions for modifications that might be relevant to your particular experiment. Culture media is not an ideal sort buffer for two reasons: the pH regulation fails under normal atmosphere causing the media to become basic and the calcium chloride in most culture medias is not compatible with the phosphate component of the instrument sheath buffer (the Basic Sorting Buffer without additional protein) leading to precipitation of calcium phosphate crystals. Following the suggested recipes below will help maximize the recovery and viability of your sorted cells.

Basic Sorting Buffer

- 1x Phosphate Buffered Saline (Ca/Mg++ free)
- 2.5mM EDTA
- 25mM HEPES, pH 7.0
- 1% Fetal Bovine Serum (Heat-Inactivated)
- 1% Pen-Strep
- 0.2um filter sterilize, store a 4°C

For Clean Lymphoid Cells: The buffer can be simplified to HBSS with 1% FBS. The additional cations in the recipe promote better viability. Since these cells are not prone to clump, the lack of EDTA is not a problem.

For Sticky Cells: Raise the concentration of the EDTA to 5mM and use FBS that has been dialyzed against Ca/Mg++ free PBS. Some activated cells become clumpy and the chelators (EDTA) help reduce cation-dependent cell to cell adhesion.

For Adherent Cells: In order achieve good single cell preparations, one must start at the moment of detaching your cells from the plate. Typically, the trypsin (or other detachment buffer) is quenched with culture media or a PBS/FBS buffer. This is problematic because it reintroduces the cations that facilitate the cells reattaching to the plate (or each other). One must use a cation-free FBS buffer in order to stop the detachment. Additionally, the level of EDTA can be increased if necessary (but too much EDTA can be deleterious).

For Samples with High Percentage of Dead Cells: If there are a large number of dead cells in the prep, it is likely that there is soluble DNA from the dead cells that will come out of solution. This DNA will start to coat the cells and lead to severe clumping. The addition of 10U/mL DNAase II to the buffer recipe will help reduce DNA associated clumpiness.

These suggestions should help to optimize sample preparation for both enhanced viability and enhanced recovery. It may require some more comprehensive modifications evolving from these simplistic guidelines.