Cell Sorting Buffers

Basic

1XPBS (Ca/Mg++free)

1mM EDTA

25mM HEPES pH7.0

1% Fetal Bovine Serum (Heat-Inactivated)

.2um filter sterilize, store at 4C

Variations

Sticky Cells- Increase concentration of EDTA to 5mM and use FBS that has been dialyzed against Ca/Mg++ free PBS.

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)

Samples with Lots of Dead/Tissue- Add 10U/ml DNAse II to the buffer