Sample Guidelines for Cell Sorting

- Samples suppose to be single cell suspension only (i.e. no clumps). Use all applicable Sample Preparation Tips
- If you have extensive cell death in the sample, add DNAse (see Sample Preparation Tips). It will not only decrease clump formation in the sample, but also will decrease viscosity of the single cell suspension buffer, by digestion of the free DNA. Sort purity and yield could go substantially up after DNAase treatment
- Add the 10-25mM of HEPES, pH range 6.8 - 8.2 (for example: HEPES buffer solution 1M in H_2O, Sigma-Aldrich Co., Cat# 83264-100ML-F) to your buffer. Addition of HEPES will significantly increase the buffer capacity of the original sample buffer. Buffer capacity of the common phosphate and carbonate buffers gets compromised by high pressure within the instrument during the cell sorting procedure.
- Provide **compensation controls**:
  - If **cells** are used as single color controls
    - Unstained cells
    - Titration of the Dead Cell Exclusion dye on the "partially killed" cells
    - All separate single color stained cell controls
      - Preferable concentration of the controls is around 1 x 10^6cells/ml, volume 0.5-1.0 ml
  - If **beads** are used as single color controls
    - Unstained Cells
      - Preferable concentration of unstained cell control is around 1 x 10^6cells/ml, volume 0.5-1.0 ml
    - Unstained beads
    - All separate single color stained bead controls
      - Concentration and bead volume are based on the protocol from Bead Compensation Controls
- Provide **gating controls** - Fluorescent Minus One (FMO) controls, as proper way to evaluate the background in certain channel and set sorting gates appropriately
- Sample concentration for the sort is 1-20 x 10^6cells/ml (dependent on the cell type and the nozzle size)
- Provide extra 15 ml of the buffer you used for the cell samples
- Provide 1ug/ml stock solution (1ml) of the non-fixable Dead Cell Exclusion dye
- Bulk Sort - Provide collection tubes for the sorted cells. Tube size depends on the expected number of post-sorted cells. *Note: Concentration of post-sorted cells is between 0.3 - 1.0 x 10^6cells/ml and depends on the nozzle size*
  - Tube types:
    - 12 x 75 mm Tubes (up to 4-way sort)
      - Polypropylene - best choice
        - sterile with snap cap (Cat#1495911A, Fisher Scientific; or Cat#352063, BD Biosciences)
      - Polystyrene - poorer choice, but still possible
        - non-sterile (Cat#352008, BD Biosciences)
        - sterile with the cap (Cat#352054, BD Biosciences)
    - 15 ml Tubes - any type (up to 2-way sort)
  - Tube pre-treatment:
    - In order to prevent cells sticking to the sides of the tubes, pre-coat the tubes, filling them with 10x BSA solution (1%)
    - Keep filled tubes inverted for at least 30' prior sort
  - Solution to sort into:
    - TRIzol LS reagent (for RNA extraction. Volume of TRIzol LS:Cells=3:1. For example, 750ul of TRIzol LS + 250ul of sorted cells)
    - PBS-based buffer
    - PBS/HEPES/BSA (see buffer suggestions)
    - Any specific solution (for example, PCR mix)
    - none

- Tube special characteristics depend on the sorting purpose:
  - For sterile sort tubes should be also sterile
  - For RNA work tubes should be RNAse free
  - For Western blot tubes should not be treated with external protein

Sort using ACDU (automated cell deposition unit):

- Receptacles:
  - Multiwell plates:
    - 96 well plate
    - 24 well plate
  - Microscope slides
- Solution to sort into - depends on the purpose of the sort:
  - Complete medium for cell growth
  - TRIzol LS reagent (for RNA extraction)
  - PCR mix
  - Etc.
- Sort setup specifics:
  - Day before the sort FCF-Berg Staff should be provided with several empty plates (or other receptacles) of the type planned to be used for the sort.

Plates (or other receptacles) for the cell sort should be brought to FCF-Berg at the time specified by FCF-Berg Staff.