New York Blood Center

# Sample preparation guide for sorting

- Test your antibody and fluorophore combination before booking a sort.
- Cells must be in a single cell suspension.
- Filter the sample just prior to sorting in order to remove cell clumps. Use the 5 ml Falcon polystyrene round-bottom tubes with cell-strainer cap (cat# 352235, 35µm nylon mash) for this purpose.
- Bring the cells in $Mg/Ca^{++}$ free buffer, containing 01-1% of BSA or 1-2% of dialyzed FBS. In order to prevent cell clumping during the sort, add EDTA (up to 5mM, prevents cation dependent cell to cell adhesion) and/or DNAse (up to 50µg/ml, prevents cell clumping due to a cell death). The cells can also be brought in Phenol Red free media.
- Use Dead Cell Exclusion dyes to eliminate dead cell.
- Sample cell concentration is cell type and nozzle size dependent - recommended concentration 5 -10x$10^6$cells/ml.
- Cells can be collected into:
  - 5ml (12x75mm) and 15 ml tubes
  - 6, 24 and 96 well plate
  - Slides
- Collection tube size/ number: a rule of thumb is that with 70µm nozzle we can put up to 3 million droplets in the 12x75mm tube. With 100µm nozzle we can put up to 1 million droplets in 12x75mm tube.
- In order to get a better recovery, pre-coat the collection tubes by filling them with media+serum or serum only for at least 30' prior to sorting.
- Solution to sort into:
  - PBS based buffer containing FBS or BSA
  - Complete media for cell growth with 10-20% FBS
  - TRIzol reagent ( for RNA extraction)
  - Etc.
- Provide compensation controls and gating controls.