Sample Preparation Guideline

**Analyzers (Calibur, CantoII, Fortessa, LSRII)**

1) Tube: Falcon polystyrene (Fisher #: 352008)
2) Concentration: 3x10^6 cells/ml, minimum volume: 400ul

**Sorter**

**Coating the collection tube (applies to both Influx and Aria)**

1) All collection tube preferably to be made from polypropylene instead of polystyrene.
2) The day before the sort, fill the collection tube to 90% full (eppendorf, 5ml, 15ml, 50ml, etc) with serum containing medium and place the collection tube with medium in the 37°C incubator.
3) If you are working with serum free medium, you may substitute the serum with 0.1% BSA (final concentration).
4) Please coat extra collection tubes in case you have more cells than expected.
5) On the day of sort, decant as much coating reagent as possible and put in the desired volume of collection medium.

**Influx**

1) Tube: Polypropylene (NOT polystyrene tube used in Calibur/Canto/LSRII) (Fisher #: 352063)
2) Concentration: 20x10^6 cells/ml in 5ml tube with less than 4ml/tube, minimum volume: 500ul.
3) Serum: No more than 2%
4) Sticky cells (especially cells isolated from tissue or adherent cells): add 10 units (or 100mg) of DNAse per ml of sample (Sigma #: D-4513), and/or with 1mM EDTA.
5) Sterile sort: 10X fungazone and antibiotics may be added in the collection tube
6) Clump removal: Cells MUST be filtered through the cell strainer RIGHT BEFORE the sort (Fisher #: 352235)
7) Collection tubes/plate: 1.5ml eppendorf, 5ml polypropylene tube, 15ml and 50ml tube with medium that best enhances the cell viability; 6, 12, 24, 48, 96-wells plate. Two ways sort: 5ml, 15ml, 50ml tube, 1.5ml eppendorf; Three way sort: two 5ml tube (or two eppendorf) and one 50ml; four way sort: 5ml tube or eppendorf.
Aria

1) Tube: Cell strainer polystyrene (Fisher #: 352235) or 15ml Falcon conical (Fisher #: 352096)

2) Concentration: 20x10^6 cells/ml, minimum volume: 500ul

3) Serum: No more than 2%

4) Sticky cells (especially cells isolated from tissue or adherent cells): add 10 units (or 100mg) of DNase per ml of sample (Sigma #: D-4513), and/or with 1mM EDTA.

5) Sterile sort: 10X fungazone and antibiotics may be added in the collection tube

6) Clump removal: Cells MUST be filtered through the cell strainer RIGHT BEFORE the sort (Fisher #: 352235)

7) Collection tubes/plate: 1.5ml eppendorf, 5ml polypropylene tube and 15ml tube with medium that best enhances the cell viability; 6, 12, 24, 48, 96-wells plate. Two ways sort: 5ml, 15ml, 1.5ml eppendorf (with cap removed); four way sort: 5ml tube or eppendorf (with cap removed).