

Sample Preparation Guideline

Analyzers (Calibur, CantoII, Fortessa, LSRII)

- 1) Tube: Falcon polystyrene (Fisher #: 352008)
- 2) Concentration: 3×10^6 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 37C 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: 20×10^6 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), 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 way 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: 20×10^6 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), 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).