

Live Cell prep for sorting on Vantage

1. Cells must be in a single cell suspension. For adherent tissue culture cells, trypsinize to release from the growing surface. Stop with serum.
2. Wash cells with staining buffer (1XPBS 2% serum).
3. Centrifuge cells to pellet. Remove supernatant.
4. Resuspend in blocking agent (serum or Fc block). *This is optional, determined by cell type and nonspecific binding.*
5. Add antibody ~50 μ l/1E6cells (concentration is dependent on the specific antibody)
Titration of antibodies is recommended to get proper concentration.
6. Incubate cell in the dark at 4°C for 30-60 min.
7. Wash cells with buffer 3 times.
8. Add Secondary Antibody if necessary, repeat steps 4-6.
9. Resuspend cells to a final concentration of ~4E6/ml in sorting buffer (1XPBS 2% serum, 100Kunitz DNase I*, 2 μ g/ml PI[†])
10. Cells that are adherent or clumpy should be sent through a 40 μ m filter to remove large clumps prior to sorting. (Falcon snap cap cell strainer cat# 352235)
11. 11. Total starting cell # is dependent on how many cells are needed from each population to be sorted.
12. 12. Two populations can be sorted simultaneously and deposited into the following tube types; microcentrifuge tubes, 12X75 tubes, 15ml conical tubes.
13. 13. One population can be sorted down to one cell/well of a 96 well plate.
14. 14. Cells should be sorted into growth medium if going into culture.

* Add if using adherent cell lines to decrease clumps.

† Add PI for viability staining Note: not compatible with all dyes combinations make sure it can be used.