

The basics of staining for cell surface proteins, including direct and indirect staining, solutions, antibody titration etc. are well described in the literature. Below are some important recommendations about choosing the fluorochromes .

1. The fluorochromes must be able to absorb light from the lasers present and must emit at different wavelengths from each other so that their fluorescence signals can be distinguished. The farther apart their fluorescence peaks, the easier it will be to resolve their signals without recourse to excessive compensation.

Please see an example of fluorochromes to use for each facility's cytometer at <http://info.med.yale.edu/immuno/cytometry> under instrument name.

2. The brightest fluorochrome should be assigned to the antibody binding to the protein with lowest expression. Generally, PE and PE-based energy transfer conjugates have the brightest fluorescence. In the case of cells with high autofluorescence, allophycocyanin (APC) yields more relative brightness than PEs. The combination of PerCP and PE as labels is ideal choice for measuring a low-density antigen (PE label) on a subpopulation defined by another antigen of medium or high density (PerCP label). There is no significant spectral compensation needed between PerCP and PE. PerCP conjugates provide their optimal sensitivity with an excitation laser power below 20 mW and therefore are not recommended for use in sorting experiments on FACS Vantage SE and MoFlo.

If expression is very low, it may, however, be necessary to test antibodies conjugated to a range of different fluorochromes as conjugation procedures are variable and it is not always possible to predict brightness from photochemical principal alone.

3. Acidic buffer conditions should be avoided during the analysis of samples stained with FITC because the fluorescence of the dye and its conjugates is PH dependent.
4. Most fluorescent labels are light sensitive. To prevent artifacts, samples should be kept away from bright light.