

Guide to Choosing and Using Fluorochromes in Multicolor Flow Cytometry

Below are some BioLegend recommendations on choosing fluorochromes to give you optimal results for flow cytometry experiments.

1. Choose the brightest fluorochromes that can be used on your instrument. (To help with this, view our [Fluorochromes for Flow Cytometry and Microscopy](#) chart and our [Fluorochrome Brightness Index](#) chart) Typically, PE is always the brightest fluorochrome, except in some cases where cells have particularly high autofluorescence, APC may yield the brightest fluorescence.
2. Choose the brightest fluorochrome for your least expressed protein and the dimmest fluorochrome for your most highly expressed protein. (View our [Expression of Common Cell Surface Proteins](#) chart to assist you.) Generally, on leukocytes, CD45 is one of the most highly expressed cell surface proteins.
3. Choose fluorochromes with emissions having the least spectral overlap. Generally, the further apart the maximal emissions, the less overlap there will be, but this is fluorochrome-dependent. There are several tools available on the www to help you analyze fluorochrome spectra. Avoid using APC with Cy5-tandems on instruments in which these cannot be compensated.
4. Use tandem dyes with caution as they are prone to uncoupling, and sensitive to photobleaching and extended incubation in fixation buffers. Always use a single-stained control for any tandem dye in order to verify that the tandem has not uncoupled, monitoring the emission from the excitation partner. For example, PE/Cy5 conjugates should also be analyzed in the PE channel.
5. Avoid acidic buffer conditions with FITC labeled samples as its fluorescence is pH-dependent.
6. Avoid exposing stained samples to bright light as most fluorochromes are susceptible to photobleaching, causing them to lose fluorescence.
7. Do analyze cells as soon as possible after staining, preferably within 24 hours. Unwanted effects can occur the longer samples have to wait for analysis, such as loss of fluorescence, contamination, degradation of cells, and loss of antibody from the cell surface. While fixation can help with some of these issues, fixation can be detrimental to tandem dyes.

There are many other resources including textbooks, journal articles, and websites to help with flow cytometry applications. Be sure to view our [Guide to Flow Cytometry Resources](#).