

Multicolor Flow Cytometry

Instrument & Fluorochrome Compatibility Chart

Fluorochromes (peak emission)



- For each instrument, the standard fluorochrome detectors are shown.
- Use one fluorochrome per detector.
- Verify the laser and detector configuration of your instrument, as instruments can be configured with various optical layouts.
- For best results, use the optimal filter sets recommended for your fluorochromes of choice.
- PE-Cy5 is recommended for use on single laser instruments only.
- Additional instruments with multicolor capabilities: Cell Lab Quanta™ SC, Stratadigm S1000, Partec CyFlow® space, Partec® CyFlow® ML, iCyt Reflection®

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OVERVIEW

Multicolor flow cytometry offers a platform to gather detailed information on specific cells within a mixed population. This is especially relevant to maximize the data that can be obtained from a small or limited sample. Getting the most from a multicolor flow cytometry experiment requires some thought and planning up front in order to maximize success. It is critical to know the configuration of the cytometer, the laser lines as well as the filter sets, and also the excitation/emission spectra of the fluorochromes to be used in the experiment.

Antigen Density and Fluorochrome Selection

Fluorochromes vary with respect to the signal intensity they generate. In general, the brightest fluorochrome (most intense) should be paired with the least densely expressed target antigen. For instance, phycoerythrin (PE) and allophycocyanin (APC) should be chosen to detect the least abundant target antigens in the assay. Additionally, target antigens whose expression is discontinuous, such as activation markers and cytokines, also work best with more intensely fluorescent dyes. It is advisable to use directly fluorochrome conjugated antibodies whenever possible for multicolor flow cytometry.

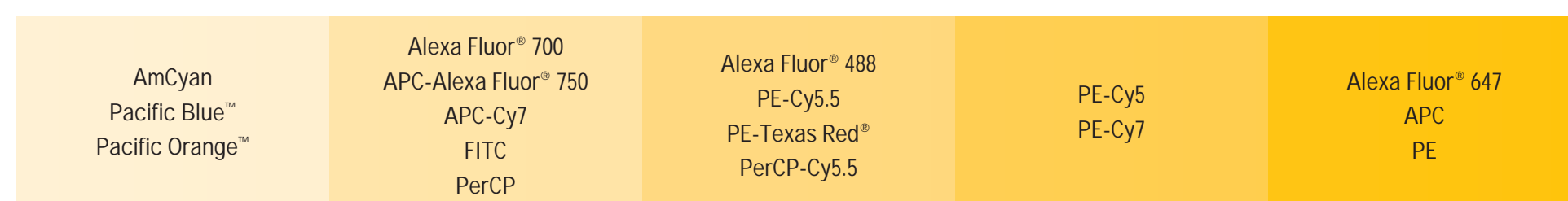
Tandem Dyes

Tandem dyes were developed in order to increase the number of target molecules that can be resolved with a single laser line. Construction of a tandem dye takes advantage of fluorescence resonance energy transfer (FRET) where a donor fluorophore is excited by the light source and transfers energy to a closely linked acceptor fluorophore that emits fluorescence at a longer wavelength. While this increases the number of probes that can be resolved on a single laser line, there are several issues to keep in mind when planning an experiment using tandem dyes. First, tandem dyes are photo-labile and must be protected from incident light. Second, most tandem dyes will emit some signal from the donor molecule so optimal compensation will be achieved by running single-color controls with the same tandem conjugate used for the experimental samples. When using tandem dyes, it is essential to comply with recommended storage and handling procedures.

eBioscience Fluorochromes:

With more fluorochrome conjugates per target than any other company it's clear why eBioscience should be your FIRST choice for multi-color flow cytometry reagents. Visit us at www.ebioscience.com for more information on all our products.

Fluorochrome Intensity Chart



Intensity →

