


Fluorochrome Conjugates For Flow Cytometry - Applications Guide

Excitation and Emission Wavelengths For Flow Cytometry



Fluorochrome	Laser (nm)	Emission (nm)
FITC	488	525
Cy3	488	565
R-PE (PE)	488	575
PE-Texas Red®	488	615
PE-Cy5 (TC ¹)	488	667
PerCP (BD ²)	488	675
PerCP/Cy5.5 (BD)	488	695
PE-Cy7	488	767
Texas Red	595	615
APC	633, 635	660
Cy5	633, 635	667
APC-Cy7	633, 635	767

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¹TC, TRI-COLOR®
²BD, Becton Dickinson Biosciences

Fluorescein (FITC)

FITC conjugates can generally be used with any flow cytometer equipped with an argon laser that emits 488 nm light. The peak emission of FITC is approximately 525 nm and is detected in the FL-1 channel. FITC conjugates can also be used for fluorescence microscopy.

R-Phycoerythrin (PE)

PE conjugates can generally be used with any flow cytometer equipped with a laser that emits 488 nm light. The peak emission of PE is approximately 575 nm and is detected in the FL-2 channel. PE is generally not recommended for routine fluorescence microscopy because it is subject to photobleaching.

PE-Cy5 (TRI-COLOR - TC)

PE-Cy5 is an energy transfer dye comprised of the indotricarbocyanine dye, Cy5, coupled to PE. PE-Cy5 is excited at 488 nm by an argon laser. The emission of PE-Cy5 begins at approximately 650 nm and peaks at 667 nm. When used on a Becton Dickinson (BD) FACScan™ or FACSCalibur™ PE-Cy5 is detected in the FL-3 channel. When used on a Coulter EPICS® XL it is detected in the FL-4 channel.

PE-Cy5 conjugates can be used with both milliwatt lasers found in the benchtop analyzers as well as with full power lasers found on the larger cell sorters. Sorters with an argon and HeNe laser must be equipped with cross beam compensation if APC and PE-Cy5 are used together.

In flow cytometric applications, the fluorescence intensity of PE-Cy5 is comparable to that of PE alone. PE-Cy5 is not recommended for routine fluorescence microscopy because it is subject to photobleaching.

PE-Cy7

PE-Cy7 is an energy transfer dye comprised of Cy7 coupled to PE. PE-Cy7 is excited at 488 nm by an argon laser. The emission of PE-Cy7 begins at approximately 700 nm and peaks at 776 nm. Its emission is detected in various channels depending upon the filter arrangement of the flow cytometer. When used on a B-D FACScan or FACSCalibur, it is detected in FL-3. On larger instruments such as the FACS Vantage™ PE-Cy7 is detected in FL-6. When used with FITC, PE and PE-Cy5 on a Coulter EPICS XL certain filters must be changed. These filters are available from Caltag or Omega Optical (www.omegafilters.com).

In flow cytometric applications, the fluorescence intensity of PE-Cy7 lies between that of FITC and PE. PE-Cy7 conjugates can be used with both benchtop analyzers and large sorters.

Texas Red (TR)

Texas Red conjugates are useful in multi-color flow cytometry with instruments equipped with a second laser that will excite TR within its absorbance range. TR can be used with fluorescent microscopes equipped with the proper filters.

PE-Texas Red (PE-TR)

PE-TR is an energy transfer dye comprised of TR coupled to PE. PE-TR is excited at 488 nm by an argon laser. The emission of PE-TR peaks at 615 nm. When used on BD instruments, it is typically detected in the FL-3 channel. When used on a Coulter EPICS XL it is typically detected in the FL-3 channel as well.

PE-TR conjugates can be used with both milliwatt lasers found in the benchtop analyzers as well as with full power lasers found on the larger cell sorters.

Allophycocyanin (APC)

Allophycocyanin conjugates are useful in multi-color flow cytometry with instruments equipped with a second laser (e.g. HeNe or red diode) that will excite the APC within its absorbance range.

APC-Cy7

APC-Cy7 is an energy transfer dye comprised of Cy7 coupled to APC. It is excited by a 633 or 635 nm emitting laser. The peak emission of APC-Cy7 is 776 nm. Its main utility is with instruments that have multiple lasers for assays requiring more than four fluorochromes.

Cyanine dyes, Cy3 & Cy5

Conjugates of Cy3 and Cy5 can be used directly in flow cytometry, but typically do not give fluorescence intensity comparable to that of PE or APC. Applications where a smaller molecular weight dye is required are more appropriate for Cy3 & Cy5 in flow cytometry. These fluorochromes are well suited for fluorescent microscopy.

Working Dilutions

As a general rule, all Caltag reagents labeled with fluorochromes can be used at 1 ug, or less, per 10⁶ cells in flow cytometric applications. Most conjugates can be diluted further without appreciable reduction in fluorescence intensity. Some reagents

must be diluted further for optimum staining. It is recommended that the investigator determine the dilution that is optimum for each assay.

Flow Cytometers and CALTAG Fluorochromes

FLUOROCHROME MACHINE	FITC	R-PE	PE-TR	PE-Cy5	PE-Cy7	APC	APC-Cy7
Becton Dickinson:							
FACScan™ (1 laser)							
FACSCalibur™ (1 laser)							
FACSCalibur (2 lasers)							
FACStar Plus™ (argon laser)*							
FACSSstar Plus™ (> 1 laser)*							
FACS Vantage™ (argon laser)*							
FACS Vantage (> 1 laser)*							
Coulter:							
EPICS® Profile (argon laser)							
EPICS XL (argon laser)							
EPICS Elite (argon laser)							
EPICS Elite (> 1 laser)							
Cytomation:							
Mo-Flo®							
Potential Combinations:							
2 color/argon laser							
3 color/argon laser							
4 color/argon laser							
4 color/>1 laser *							
4 color/>1 laser *							
5 color/>1 laser *							
5 color/>1 laser *							
6 colors/>1 laser *							

* The fluorochrome combinations that can be used depend upon the lasers, filters and compensation circuitry available on each particular instrument.



Indicates the instrument or application where fluorochrome is commonly used.