FLUOROCHROME CONJUGATES FOR FLOW CYTOMETRY - APPLICATIONS GUIDE

Excitation and Emission Wavelengths for Flow Cytometry

Fluorochrome	Laser (nm)	Emission (nm)
Alexa Fluor®488	488	519
FITC	488	525
Cy™3	488	570
R-PE	488	575
PE-Texas Red®	488	615
PE-Alexa Fluor 610	488	628
PE-Alexa Fluor 647	488	668
PE-Cy5 (TC)	488	670
PE-Cy5.5	488	694
PE-Alexa Fluor 700	488	723
PE-Cy7	488	767
Texas Red	595	615
APC	633 / 635	660
Alexa Fluor 647	633 / 635	668
Cy5	633 / 635	670
APC-Cy5.5	633 / 635	694
APC-Cy7	633 / 635	767

Working Dilutions

As a general rule, all Caltag reagents labeled with fluorochromes should be used at ≤ 1 µg per 10^6 cells in flow cytometric applications. Some reagents must be diluted for optimal staining. It is recommended that the investigator determine the dilution that is optimal for each assay.

Alexa Fluor® 488

Alexa Fluor 488 conjugates can be used with any flow cytometer equipped with an argon laser that emits at 488 nm. The peak emission of Alexa Fluor 488 is 519 nm which is measured in the FL1 channel. Due to its extraordinary photostability, this fluorochrome is also highly suitable for fluorescence microscopy. Further, unlike other fluorochromes with similar spectral properties, Alexa Fluor 488 is stable over a broad pH range, pH 4 to 10.

Fluorescein (FITC)

FITC conjugates can be used with any flow cytometer equipped with an argon laser that emits at 488 nm. The peak emission of FITC is at 525 nm which is measured in the FL1 channel. FITC conjugates can also be used for fluorescence microscopy.

CyDye™ Fluors, Cy™3 & Cy5

Conjugates of Cy3 and Cy5 can be used directly in flow cytometry, but typically do not exhibit fluorescence intensity comparable to that of PE or APC. The CyDye fluors are preferred for flow cytometric applications requiring a smaller molecule dye. These fluorochromes are well suited for fluorescence microscopy.

R-Phycoerythrin (R-PE, PE)

PE conjugates can be used with any flow cytometer equipped with a laser that emits at 488 nm. The peak emission PE is at 575 nm which is normally measured in the FL2 channel. Because it is susceptible to photobleaching, PE is generally not recommended for conventional fluorescence microscopy; however, conjugates of this fluorochrome are gaining widespread use in laser scanning confocal microscopy.

PE-Texas Red (PE-TR)

PE-TR, also know as ECD®, is an energy transfer dye comprised of Texas Red® (TR) coupled to R-Phycoerythrin. PE-TR is excited at 488 nm by an argon laser. The emission of PE-TR peaks at 615 nm. PE-TR is typically detected in the FL3 channel.

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.

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PE-Alexa Fluor 610

PE-Alexa Fluor 610 is an energy transfer dye comprised of Alexa Fluor 610 coupled to R-Phycoerythrin. This dye has similar spectral properties to PE-TR but delivers a higher quantum yield. PE-Alexa Fluor 610 is excited at 488 nm by an argon laser. The fluorescence emission of PE-Alexa Fluor 610, which peaks at 628 nm, is typically measured in the FL3 channel on both Becton Dickinson (BD) and Beckman Coulter® instruments. However, for optimal use on the Coulter EPICS® XL™, filter modifications are strongly recommended. The necessary filters are available from Caltag or Omega Optical, Inc. Please contact Caltag's Technical Services Department for additional information.

PE-Alexa Fluor 610 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.

PE-Alexa Fluor 647

PE-Alexa Fluor 647 is an energy transfer dye comprised of Alexa Fluor 647 coupled to R-Phycoerythrin. PE-Alexa Fluor 647 is excited at 488 nm with an argon laser. The emission of PE-Alexa Fluor 647 peaks at 668 nm. When used on a BD FACScan™ or FACSCalibur™, it is measured in the FL3 channel. When used on a Coulter EPICS XL it is usually measured in the FL4 channel.

PE-Alexa Fluor 647 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 simultaneously equipped with argon and HeNe lasers must have cross beam compensation circuitry if APC and Alexa Fluor 647 are to be used together.

PE-Cy5 (TRI-COLOR®, TC)

PE-Cy5 is an energy transfer dye comprised of Cy5 coupled to R-Phycoerythrin. PE-Cy5 is excited at 488 nm with an argon laser. The emission of PE-Cy5 peaks at 670 nm. When used on a BD FACScan or FACSCalibur, it is measured in the FL3 channel. When used on a Coulter EPICS XL it is usually measured in the FL4 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 simultaneously equipped with argon and HeNe lasers must have cross beam compensation circuitry if APC and PE-Cy5 are to be used together. PE-Cy5 is not recommended for conventional fluorescence microscopy because it is subject to photobleaching.

PE-Cy5.5

PE-Cy5.5 is an energy transfer dye comprised of Cy5.5 coupled to R-Phycoerythrin. PE-Cy5.5 is excited at 488 nm with an argon laser. The emission of PE-Cy5.5 peaks at 694 nm. When used on a BD FACScan or FACSCalibur, it is measured in the FL3 channel. When used on a Coulter EPICS XL it is usually measured in the FL4 channel.

PE-Cy5.5 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. Although the cross-over fluorescence of PE-Cy5.5 with APC is negligible, it is recommended that instruments on which these fluorochromes will be used simultaneously be equipped with cross beam compensation circuitry. PE-Cy5.5 is not recommended for routine fluorescence microscopy because it is subject to photobleaching.

PE-Alexa Fluor 700

PE-Alexa Fluor 700 is an energy transfer dye comprised of Alexa Fluor 700 coupled to R-Phycoerythrin. PE-Alexa Fluor 700 is excited at 488 nm with an argon laser. The emission of PE-Alexa Fluor 700 peaks at 723 nm. The channel in which its fluorescence is measured will depend on the filter arrangement of the flow cytometer being used. When used on a BD FACScan or FACSCalibur, it is detected in the FL3 channel. 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, Inc. Please contact Caltag's Technical Services Department for additional information.

PE-Alexa Fluor 700 conjugates can be used with both benchtop analyzers and large sorters. However, it should be noted that Alexa Fluor 700-containing tandem dyes are particularly sensitive to photo-induced degradation. Extreme caution should be exercised to avoid light exposure throughout the staining process and during analysis.

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PE-Cy7

PE-Cy7 is an energy transfer dye comprised of Cy7 coupled to R-Phycoerythrin. PE-Cy7 is excited at 488 nm with an argon laser. The emission of PE-Cy7 peaks at 767 nm. The channel in which its fluorescence is measured will depend on the filter arrangement of the flow cytometer being used. When used on a BD FACScan or FACSCalibur, it is measured in the FL3 channel. When used with FITC, PE and PE-Cy5 on a Coulter EPICS XL, certain filters must be changed. These filters are available from Caltag, Omega Optical, Inc., or Chroma Technology Corp. Please contact Caltag's Technical Services Department for additional information.

PE-Cy7 conjugates can be used with both benchtop analyzers and large sorters. However, it should be noted that Cy7-containing tandem dyes are particularly sensitive to photo-induced degradation. Extreme caution should be exercised to avoid light exposure throughout the staining process and during analysis.

Texas Red

Texas Red conjugates are useful in multi-color flow cytometry with instruments equipped with a laser that will excite TR within its absorbance range. TR has its peak emmision at 615 nm and can be used with fluorescence microscopes equipped with the proper filters.

Allophycocyanin (APC)

Allophycocyanin conjugates are useful in multi-color flow cytometry with instruments equipped with a HeNe or red diode laser that emit at 633 and 635, respectively. The fluorescence emission of APC peaks at 660 nm.

Alexa Fluor 647

Alexa Fluor 647 presents an alternative to APC as well as Cy5. Alexa Fluor 647 conjugates can be used in multi-color flow cytometry with instruments equipped with a HeNe or red diode laser that emit at 633 and 635, respectively. The emission of Alexa Fluor 647 peaks at 668 nm. Like the other Alexa Fluor dyes, Alexa Fluor 647 exhibits uncommon photostability, making it an ideal choice for use in fluorescence microscopy.

APC-Cy5.5

APC-Cy5.5 is an energy transfer dye comprised of Cy5.5 coupled to APC. APC-Cy5.5 is excited at 633, 635 nm with a HeNe or red diode laser. The emission of APC-Cy5.5 peaks at 694 nm. The dye's main utility is with instruments that have multiple lasers for assays requiring more than four fluorochromes.

APC-Cy7

APC-Cy7 is an energy transfer dye comprised of Cy7 coupled to APC. It is excited at 633, 635 nm with a HeNe or red diode laser. The emission of APC-Cy7 peaks at 767 nm. The dye's main utility is with instruments that have multiple lasers for assays requiring more than four fluorochromes.