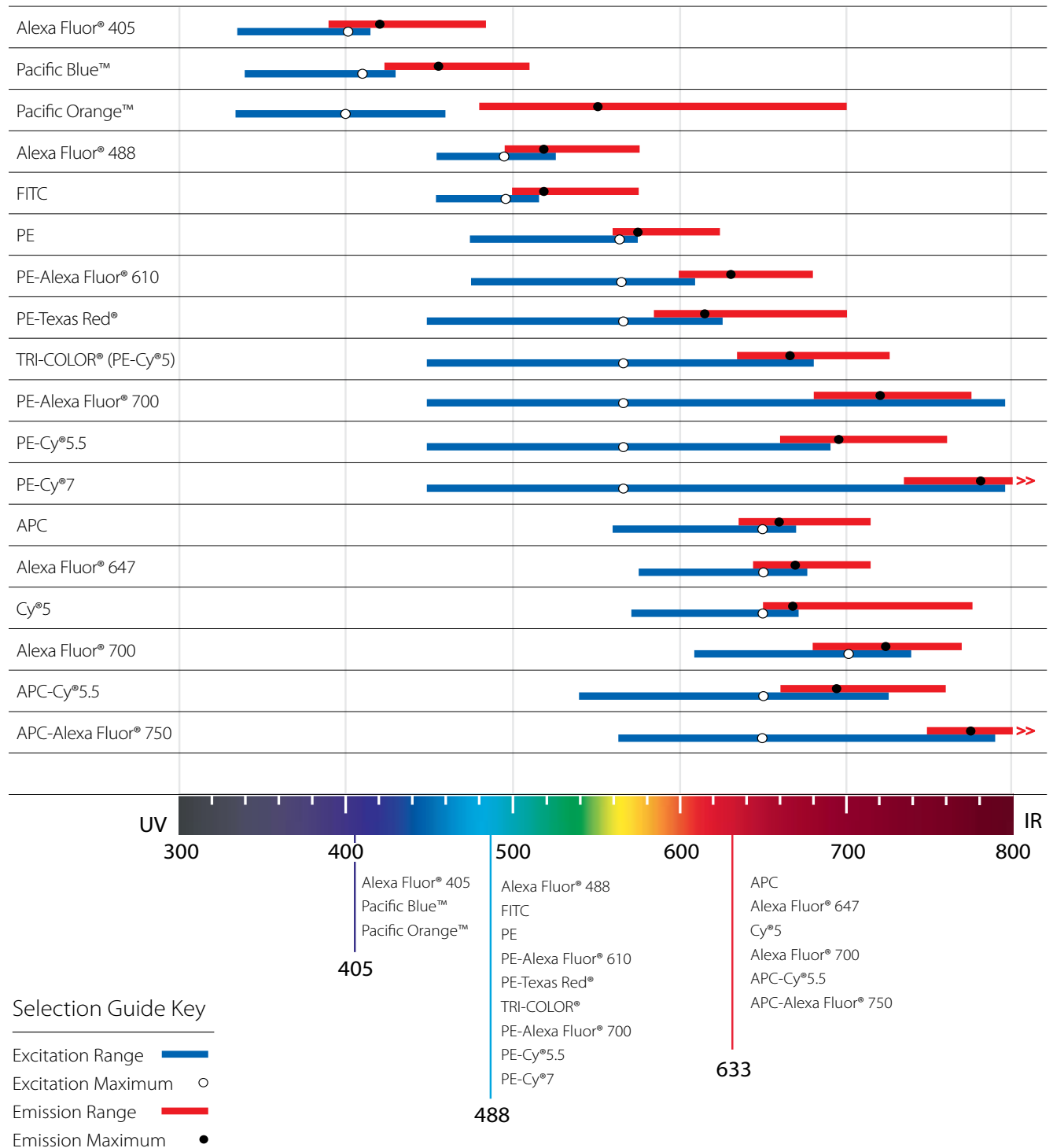


Excitation and Emission Wavelengths for Fluorochrome Conjugates for Flow Cytometry



Important Licensing Information

These products are intended for a variety of uses (RUO, IVD, ASR), commercial use is specifically excluded. If your intended use is not indicated, please refer to the product data sheet at www.invitrogen.com

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.
Pacific Blue™ and Pacific Orange™	Pacific Blue™ and Pacific Orange™ conjugates are both excited with the 405 nm spectral line of the violet laser and emit at 455 and 551 nm, respectively, facilitating two-color analysis using 405 nm excitation and multiparameter analysis using the other flow cytometer channels.
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 & Cy®5	Conjugates of Cy®3 and Cy®5 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 of 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-Texas Red®, also known as ECD®, is an energy transfer dye composed of Texas Red® coupled to R-phycoerythrin. PE-Texas Red® is excited at 488 nm by an argon laser. The emission of PE-Texas Red® peaks at 615 nm. PE-Texas Red® is typically detected in the FL3 channel. PE-Texas Red® conjugates can be used with milliwatt lasers found in benchtop analyzers as well as with full-power lasers found on larger cell sorters.
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 spectral properties similar to PE-Texas Red® 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 Omega Optical, Inc. Please contact Technical Services for additional information. PE-Alexa Fluor® 610 conjugates can be used with milliwatt lasers found in benchtop analyzers as well as with full-power lasers found on larger cell sorters.
PE-Cy®5 (TRI-COLOR®)	PE-Cy®5 is an energy transfer dye composed of Cy®5 coupled to R-phycoerythrin. PE-Cy®5 is excited at 488 nm with an argon laser. The emission of PE-Cy®5 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-Cy®5 conjugates can be used with milliwatt lasers found in benchtop analyzers as well as with full-power lasers found on larger cell sorters. Sorters simultaneously equipped with argon and He-Ne lasers must have cross-beam compensation circuitry if APC and PE-Cy®5 are to be used together. PE-Cy®5 is not recommended for conventional fluorescence microscopy because it is subject to photobleaching.
PE-Cy®5.5	PE-Cy®5.5 is an energy transfer dye composed of Cy®5.5 coupled to R-phycoerythrin. PE-Cy®5.5 is excited at 488 nm with an argon laser. The emission of PE-Cy®5.5 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-Cy®5.5 conjugates can be used with milliwatt lasers found in benchtop analyzers as well as with full-power lasers found on larger cell sorters. Although the cross-over fluorescence of PE-Cy®5.5 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-Cy®5.5 is not recommended for routine fluorescence microscopy because it is subject to photobleaching.

Important Product Classifications

ASR: Analyte Specific Reagent. Analytical performance characteristics are not established for *in vitro* diagnostic use. RUO: For Research Use Only. Not for therapeutic or *in vitro* diagnostic use. GPR: General Purpose Reagent. For *in vitro* diagnostic use. IVD: *In vitro* diagnostic product.

PE-Alexa Fluor® 700	PE-Alexa Fluor® 700 is an energy transfer dye composed 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-Cy®5 on a Coulter EPICS XL, certain filters must be changed. These filters are available from Omega Optical, Inc. Please contact Technical Services 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.
PE-Cy®7	PE-Cy®7 is an energy transfer dye composed of Cy®7 coupled to R-phycoerythrin. PE-Cy®7 is excited at 488 nm with an argon laser. The emission of PE-Cy®7 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-Cy®5 on a Coulter EPICS XL, certain filters must be changed. These filters are available from Omega Optical, Inc., or Chroma Technology Corp. Please contact Technical Services for additional information. PE-Cy®7 conjugates can be used with both benchtop analyzers and large sorters. However, it should be noted that Cy®7-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 multicolor flow cytometry with instruments equipped with a laser that will excite Texas Red® within its absorbance range. Texas Red® has its peak emission at 615 nm and can be used with fluorescence microscopes equipped with the proper filters.
Allophycocyanin (APC)	Allophycocyanin conjugates are useful in multicolor flow cytometry with instruments equipped with a He-Ne or red diode laser that emits at 633 or 635 nm, respectively. The fluorescence emission of APC peaks at 660 nm.
Alexa Fluor® 647	Alexa Fluor® 647 presents an alternative to APC as well as Cy®5. Alexa Fluor® 647 conjugates can be used in multicolor flow cytometry with instruments equipped with a He-Ne or red diode laser that emits at 633 or 635 nm, 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.
Alexa Fluor® 700	With an absorption maximum at 702 nm, the isomer-free Alexa Fluor® 700 dye can be excited with a 633 nm far-red diode laser. Alexa Fluor® 700 dye provides bright fluorescence emission with a peak at ~723 nm. This bright and photostable single, small-molecule dye is an excellent alternative to APC-Cy®5.5 tandem conjugates.
APC-Cy®5.5	APC-Cy®5.5 is an energy transfer dye composed of Cy®5.5 coupled to APC. APC-Cy®5.5 is excited at 633, 635 nm with a He-Ne or red diode laser. The emission of APC-Cy®5.5 peaks at 694 nm. The dye's main utility is with instruments that have multiple lasers for assays requiring more than four fluorochromes.
APC-Alexa Fluor® 750	APC-Alexa Fluor® 750 is an energy transfer dye composed of Alexa Fluor® 750 coupled to APC. It is excited at 633, and 635 nm with a He-Ne or red diode laser. The emission of APC-Alexa Fluor® 750 peaks at 775 nm. APC-Alexa Fluor® 750 is completely interchangeable with APC-Cy®7. In addition to being brighter than APC-Cy®7, APC-Alexa Fluor® 750 also has much lower compensation versus APC than APC-Cy®7.

Please contact Technical Service to discuss optimal fluorochrome conjugates for your flow cytometer, or refer to the instrument manual to find the recommended fluorochrome conjugate for a channel.

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