Fluorophores for Flow Cytometry

BioLegend®

04-0025-00

Pacific Blue™ is a hydroxycoumarin-based fluorophore, generally considered to be dimmer than most other fluorophores. It is recommended for highly expressed antigens. Brilliant Violet 421™ is a brighter alternative for weakly expressed antigens. Ex_{max} = 401 nm, Em_{max} = 455 nm

Brilliant Violet 421™ is a novel organic fluorescent polymer capable of extremely bright signal and high photostability. It is an excellent choice for flow cytometry and microscopy. It is not recommended for use with Pacific Blue™. Ex_{max} = 405 nm, Em_{max} = 421 nm

Brilliant Violet 510™ is a novel polymer with excellent signal-to-noise, excited by the violet laser. It can provide dramatic improvements over existing fluorophores emitting in this range, such as Pacific Orange™ and Horizon™ V500. Ex_{max} = 405 nm, Em_{max} = 510 nm

Brilliant Violet 785™ is a novel molecule based on the Brilliant Violet 421™ polymer core. It provides further options for the violet laser and is a better alternative to nanocrystals for intracellular flow cytometry.[1] Ex_{max} = 405 nm, Em_{max} = 785 nm

Brilliant Violet 711™ is a novel molecule based on the Brilliant Violet 421™ polymer core. It provides a much brighter alternative to eFluor® 700NC for multicolor flow on the violet laser and is a better alternative to nanocrystals for intracellular flow cytometry.[1] Ex_{max} = 405 nm, Em_{max} = 711 nm

Pacific Blue™ 1

Brilliant Violet 421™ 5

Brilliant Violet 785™ 3

Violet Laser 405 nm

Brilliant Violet 510™ 4

Brilliant Violet 711™ 4

Brilliant Violet 570™ 3

Brilliant Violet 650™ 4

Brilliant Violet 605™ 5

Brilliant Violet 650™ is a novel organic fluorescent polymer. It provides a much brighter alternative to eFluor® 650 for multicolor flow on the violet laser and is a better alternative to nanocrystals for intracellular flow cytometry.[1] Ex_{max} = 405 nm, Em_{max} = 646 nm

Brilliant Violet 605™ is a novel organic fluorescent polymer. It provides a much brighter alternative to eFluor® 605 for multicolor flow on the violet laser and is a better alternative to nanocrystals for intracellular flow cytometry.[1] Ex_{max} = 405 nm, Em_{max} = 603 nm

Brilliant Violet 570™ is a novel organic fluorescent polymer. It provides a much brighter alternative to Pacific Orange™ for multicolor flow on the violet laser and is a better alternative to nanocrystals for intracellular flow cytometry.[1] Ex_{max} = 405 nm, Em_{max} = 570 nm

PE/Cy5 is a tandem conjugate of PE and the cyanine dye, Cy5. Similar to PE, it is bright for flow cytometry, but is sensitive to photobleaching and not recommended for microscopy. Cy5 has been known to non-specifically bind to Fc receptors. On some instruments, it is not recommended for use with APC, due to their overlapping emissions. Ex_{max} = 496 nm, Em_{max} = 667 nm

PE/Texas Red-X is a tandem conjugate of PE and a sulforhodamine dye, Texas Red-X. Similar to PE, it is bright for flow cytometry. Due to its unique emission range, falling between PE and PE/Cy5, it is an excellent choice for expanding multicolor options on the 532 or 561 nm lasers, in addition to the 488 nm laser. Ex_{max} = 565 nm, Em_{max} = 613 nm

PE/Cy5 5

PE/Texas Red-X 4

Green/Yellow-Green Laser 532/561 nm

R-Phycoerythrin (PE) is a pigment derived from red algae. It is a large 240 kD protein that contains 23 phycoerthrobilin chromophores per molecule. It is extremely bright for flow cytometry, but poor photostability makes it unsuitable for microscopy. PE and its tandems are also sensitive to denaturation by fixation. Because of its wide excitation range, it can be excited by the 488, 532, and 561 nm lasers. Ex_{max} = 496 nm, Em_{max} = 578 nm

PE 5

PE/Cy7 4

PE/Cy7 is a tandem conjugate of PE and the cyanine dye, Cy7. Similar to PE, it is bright for flow cytometry, but is sensitive to photobleaching and not recommended for microscopy. This tandem is particularly sensitive to light exposure and prolonged fixation. Ex_{max} = 496 nm, Em_{max} = 785 nm

PerCP is a naturally-derived carotenoid pigment found in photosynthetic dinoflagellates. It is not recommended for high powered lasers >25 mW due to its sensitivity to photobleaching. PerCP has overlapping emission with PE/Cy5, so these should not be used on the same laser. Ex_{max} = 482 nm, Em_{max} = 678 nm

PerCP/Cy5.5 is a tandem conjugate of PerCP and the cyanine dye, Cy5.5. Unlike PerCP, it is quite photostable and can be used with high powered lasers. Of all the tandems, it is the most photostable and resistant to degradation by fixation. PerCP and PerCP/Cy5.5 have significant overlap and are not recommended for use together. Ex_{max} = 482 nm, Em_{max} = 695 nm

Alexa Fluor® 488 is a fluorescent molecule with exceptional photostability. For flow cytometry, it has very similar brightness and emission properties compared to FITC, but is advantageous for microscopy applications. Ex_{max} = 495 nm, Em_{max} = 519 nm

FITC (fluorescein isothiocyanate) is a small synthetic organic fluorophore with moderate brightness, suitable for flow and microscopy applications. It is sensitive to pH changes. It cannot be used with Alexa Fluor® 488. Ex_{max} = 494 nm, Em_{max} = 520 nm

7-AAD (7-amino-actinomycin D) has a high DNA binding constant and is efficiently excluded by intact cells. It is useful for DNA analysis and dead cell discrimination during flow cytometric analysis. When excited by 488 laser light, 7-AAD fluorescence is detected in the far red range of the spectrum (650 nm long-pass filter). Ex_{max} = 546 nm, Em_{max} = 647 nm

Propidium iodide (PI) is a fluorescent dye that binds to DNA. When excited by 488 nm laser light, it can be detected with a 562-588 nm band pass filter. It is commonly used in evaluation of cell viability or DNA content in cell cycle analysis by flow cytometry. Ex_{max} = 305, 540 nm, Em_{max} = 620 nm

PerCP 2

PE/Texas Red-X 4

PerCP/Cy5.5 3

PE 5

Blue Laser 488 nm

PE/Cy5 5

Alexa Fluor® 488 3

PE/Cy7 4

FITC 3

Propidium Iodide 5

7-AAD 5

Allophycocyanin (APC) is a 105 kD molecule derived from blue-green algae that has 6 phycocyanobilin chromophores per molecule. It is bright and particularly useful for flow cytometry but not microscopy. It should not be used together with Alexa Fluor® 647 due to their overlapping emissions. Ex_{max} = 650 nm, Em_{max} = 660 nm

APC/Cy7 is a tandem of APC and the cyanin dye, Cy7. It is particularly sensitive to light and fixation and should be handled with care to avoid light exposure. Ex_{max} = 650 nm, Em_{max} = 785 nm

Alexa Fluor® 647 is spectrally equivalent to APC, but has much better photostability, making it ideal for microscopy applications. While generally not as bright as APC, it is also a good choice for flow cytometry. Ex_{max} = 650 nm, Em_{max} = 668 nm

Alexa Fluor® 700 Although it is a dim molecule, Alexa Fluor® 700 expands the choices for the red laser, fitting nicely between APC and APC/Cy7. Ex_{max} = 696 nm, Em_{max} = 719 nm

APC 5

Red Laser 633 nm

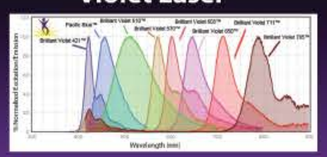
APC/Cy7 2

Alexa Fluor® 647 4

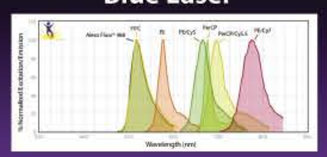
Alexa Fluor® 700 2

biolegend.com

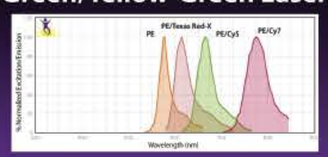
Violet Laser



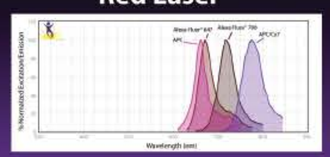
Blue Laser



Green/Yellow-Green Laser



Red Laser



1. biolegend.com/brilliantviolet. Select fluorophore of interest and click "Beta Testing".

Interactive Flow Cytometry Instrument Guide: **biolegend.com/instrument_guide**

Our technical service team is available to help with any questions you may have on multicolor flow cytometry.

Technical Service

Phone Toll-Free (US & Canada)**1-877-273-3103**

Phone (International): **1-858-768-5801**

Brightness Index:

indicated below each fluorophore name

1 2 3 4 5

The Fluorophore Brightness Index Score is a relative indication of fluorescence intensity above the background for each fluorophore antibody conjugate (**1 = dim, 5 = brightest**). These values can differ depending upon the flow cytometer, instrument filters and settings, laser power, antibody clone and antigen target, f/p ratios, buffer conditions, etc.