# **BD Biosciences** Fluorochrome Reference Chart

Visit **bdbiosciences.com/colors** for detailed information about our newest fluorochromes and instrumentation.
To select your optimal combination of fluorochromes, visit **bdbiosciences.com/spectra** to use an interactive fluorescence spectrum tool.

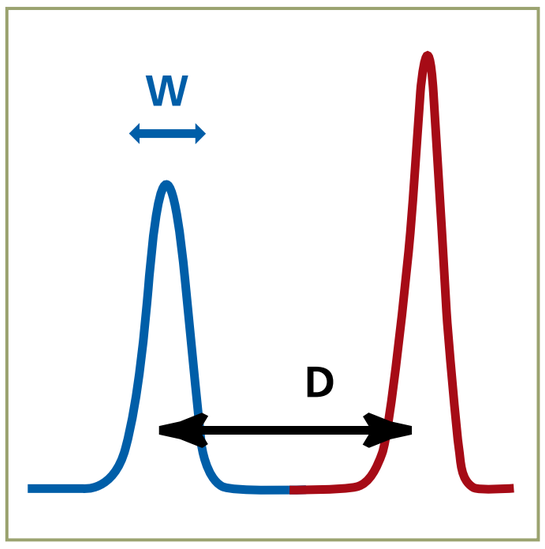
| Instrument | Excitation Laser Line (nm) | Fluorescence Channel | Fluorochromes provided by BD Biosciences | | | | |
|---|---|---|---|---|---|---|---|
| **BD Accuri™ C6** | 488 | FL1 Green | FITC | Alexa Fluor® 488 | | | |
| | | FL2 Yellow | PE | PI | | | |
| | | FL3 Red | 7-AAD | PerCP | PerCP-Cy™5.5 | PE-Cy™7 | |
| | 640 | FL4 Red | APC | Alexa Fluor® 647 | | | |
| **BD FACSCalibur™** | 488 | FL1 Green | FITC | Alexa Fluor® 488 | | | |
| | | FL2 Yellow | PE | PI | | | |
| | | FL3 Red | 7-AAD | PE-Cy™5 | PerCP | PerCP-Cy5.5 | PE-Cy7 |
| | 635 | FL4 Red | APC | Alexa Fluor® 647 | | | |
| **BD FACSVerse™*** | 488 | Green | FITC | Alexa Fluor® 488 | | | |
| | | Yellow | PE | PI | | | |
| | | Orange | BD Horizon™ PE-CF594[a] | PE-Texas Red®[a] | | | |
| | | Red | 7-AAD | PE-Cy5 | PerCP | PerCP-Cy5.5 | |
| | | Infrared | PE-Cy7 | | | | |
| | 640[b] | Red | APC | Alexa Fluor® 647 | | | |
| | | Far Red | Alexa Fluor® 700[a] | | | | |
| | | Infrared | BD APC-H7 | APC-Cy7 | | | |
| | 405[b] | Green | BD Horizon™ V500 | AmCyan | | | |
| | | Blue | Brilliant Violet™ 421 | BD Horizon™ V450 | VPD450 | Pacific Blue™ | |
| **BD FACSCanto™ II** | 488 | Green | FITC | Alexa Fluor® 488 | | | |
| | | Yellow | PE | PI | | | |
| | | Orange | BD Horizon PE-CF594[a] | PE-Texas Red®[a] | | | |
| | | Red | 7-AAD | PE-Cy5 | PerCP | PerCP-Cy5.5 | |
| | | Infrared | PE-Cy7 | | | | |
| | 633 | Red | APC | Alexa Fluor® 647 | | | |
| | | Far Red | Alexa Fluor® 700[a] | | | | |
| | | Infrared | BD APC-H7 | APC-Cy7 | | | |
| | 405[b] | Green | BD Horizon V500 | AmCyan | | | |
| | | Blue | Brilliant Violet™ 421 | BD Horizon™ V450 | VPD450 | Pacific Blue™ | |
| **BD LSRFortessa™ and Special Order BD LSRFortessa** (typical setup)[b] | 488 | Green | FITC | Alexa Fluor® 488 | | | |
| | | Yellow | PE | PI | | | |
| | | Orange | BD Horizon PE-CF594 | PE-Texas Red® | | | |
| | | Red | 7-AAD | PE-Cy5 | PerCP | PerCP-Cy5.5 | |
| | | Infrared | PE-Cy7 | | | | |
| | 532[b] or 561[b] | Yellow | PE | PI | | | |
| | | Orange | BD Horizon PE-CF594 | PE-Texas Red® | | | |
| | | Red | PE-Cy5 | | | | |
| | | Infrared | PE-Cy7 | | | | |
| | 640 | Red | APC | Alexa Fluor® 647 | | | |
| | | Far Red | Alexa Fluor® 700 | | | | |
| | | Infrared | BD APC-H7 | APC-Cy7 | | | |
| | 405 | Green | BD Horizon V500 | AmCyan | | | |
| | | Blue | Brilliant Violet™ 421 | BD Horizon™ V450 | VPD450 | Pacific Blue™ | |
| | 355 | Blue | Hoechst 33342 | | | | |
| **BD FACSAria™ III and Special Order BD FACSAria** (typical setup)[b] | 488 | Green | FITC | Alexa Fluor® 488 | | | |
| | | Yellow | PE | PI | | | |
| | | Orange | BD Horizon PE-CF594 | PE-Texas Red® | | | |
| | | Red | 7-AAD | PE-Cy5 | PerCP | PerCP-Cy5.5 | |
| | | Infrared | PE-Cy7 | | | | |
| | 561 | Yellow | PE | PI | | | |
| | | Orange | BD Horizon PE-CF594 | PE-Texas Red® | | | |
| | | Red | PE-Cy5 | | | | |
| | | Infrared | PE-Cy7 | | | | |
| | 640 | Red | APC | Alexa Fluor® 647 | | | |
| | | Far Red | Alexa Fluor® 700 | | | | |
| | | Infrared | BD APC-H7 | APC-Cy7 | | | |
| | 405 | Green | BD Horizon V500 | AmCyan | | | |
| | | Blue | Brilliant Violet™ 421 | BD Horizon™ V450 | VPD450 | Pacific Blue™ | |
| | 375[b] | Blue | Hoechst 33342 | | | | |
| **BD Influx™** | 488 | Green | FITC | Alexa Fluor® 488 | | | |
| | | Yellow | PE | PI | | | |
| | | Orange | BD Horizon PE-CF594 | PE-Texas Red® | | | |
| | | Red | 7-AAD | PE-Cy5 | PerCP | PerCP-Cy5.5 | |
| | | Infrared | PE-Cy7 | | | | |
| | 532 or 561 | Yellow | PE | PI | | | |
| | | Orange | BD Horizon PE-CF594 | PE-Texas Red® | | | |
| | | Red | PE-Cy5 | | | | |
| | | Infrared | PE-Cy7 | | | | |
| | 640 | Red | APC | Alexa Fluor® 647 | | | |
| | | Far Red | Alexa Fluor®700 | | | | |
| | | Infrared | BD APC-H7 | APC-Cy7 | | | |
| | 405 | Green | BD Horizon V500 | AmCyan | | | |
| | | Blue | Brilliant Violet™ 421 | BD Horizon™ V450 | VPD450 | Pacific Blue™ | |
| | 375 | Blue | Hoechst 33342 | | | | |

[a]*Available through laser and/or detector options.*
[b]*More laser and detector options are available through the Special Order Research Products (SORP) program.*

## Brightness of various fluorochrome conjugates

| Relative Brightness | Reagent | Filter |
|---|---|---|
| BRIGHTEST | Brilliant Violet™ 421 | 450/50 |
| | PE | 575/26 |
| | BD Horizon PE-CF594 | 610/20 |
| | PE-Cy5 | 670/14 |
| | APC | 660/20 |
| BRIGHT | PE-Cy7 | 780/60 |
| | Alexa Fluor® 647 | 660/20 |
| | PerCP-Cy5.5 | 695/40 |
| MODERATE | Alexa Fluor® 488 | 530/30 |
| | FITC | 530/30 |
| | BD Horizon V450 | 450/50 |
| | Pacific Blue™ | 450/50 |
| DIM | Alexa Fluor® 700 | 730/45 |
| | PerCP | 695/40 |
| | APC-Cy7 | 780/60 |
| | AmCyan | 525/20 |
| | BD Horizon V500 | 525/20 |
| | BD APC-H7 | 780/60 |

*Freshly isolated lymphocytes, stained with anti-human CD4 (RPA-T4) conjugated with various fluorochromes run on a BD LSR II flow cytometer. The fluorochromes were ranked based on observed stain index values. This chart is meant as a guideline of relative stain indices of various fluorochromes. Observed relative stain indices may vary depending on instrument, instrument configuration, reagents, and cell type used.*




**Stain Index = D/W**
*Resolution sensitivity (the ability to resolve a dim positive signal from background) is a function of the difference between positive and background peak means (D) and the spread of the background peak (W). The stain index is a metric that captures both of these factors.*

## **Choose a winning combination -** Guidelines for selecting reagents for multicolor flow cytometry

**1 The basics: Know your instrument**
Reagent selection starts with your instrument configuration. The lasers and detectors in your configuration dictate how well your cytometer can excite and measure a given fluorochrome, and whether you have enough detectors to read out a given combination of fluorochromes.

**2 Fluorochromes: Go for the bright**
Rank available dyes according to their intrinsic brightness on a particular instrument (when configured with a specified set of lasers and filters).

**3 Minimize spillover**
As soon as cells are stained with multiple reagents, spectral overlap (or spillover) becomes an issue. The more colors you attempt to resolve on any particular cell, the more spillover impacts sensitivity. We use compensation, an adjustment applied to all colors, to correct for spillover. For example, a cell population fluorescing only in FITC will show no PE fluorescence, on average, but will likely exhibit more spread in the PE detector after compensation than completely unstained cells.

**4 Colors and specificities: Define winning combinations**
Once the fluorochromes to be used have been defined, you can begin to match antibody specificities to particular fluorochromes. Generally, reserve the brightest fluorochromes for dim antigens, and vice versa, but avoid spillover from bright cell populations into detectors requiring high sensitivity for those populations.

**5 Tandem dyes**
APC-Cy7, and to a lesser extent, PE-Cy7, can degrade in the presence of light, fixative, and elevated temperatures so that they emit in the parent dye detector (APC or PE). By minimizing the exposure of samples to light, heat, and formaldehyde-based fixatives, this problem can be largely avoided. For more stable tandem dyes, BD now offers BD APC-H7 conjugated antibodies.

**6 Validation**
Use controls (such as fluorescence-minus-one, or FMO) to validate your selected multicolor reagent cocktail. FMO controls help define the contribution of spillover to the background in a given detector, and are therefore useful in gauging the sensitivity of that detector in the context of a certain reagent cocktail.

*For additional guidelines, visit **bdbiosciences.com/colors** to download the Application Note "Selecting Reagents for Multicolor Flow Cytometry."*

* Capable of detecting 8 colors simultaneously (4 blue laser, 2 red laser, 2 violet laser)

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

APC-Cy7: US patent 5,714,386

Brilliant Violet™ 421 is a trademark of Sirigen Group Ltd.

Cy™ is a trademark of Amersham Biosciences Corp. Cy dyes are subject to proprietary rights of Amersham Biosciences Corp and Carnegie Mellon University and are made and sold under license from Amersham Biosciences Corp only for research and in vitro diagnostic use. Any other use requires a commercial sublicense from Amersham Biosciences Corp, 800 Centennial Avenue, Piscataway, NJ 08855-1327, USA.

Pacific Blue™ is a trademark, and Alexa Fluor® and Texas Red® are registered trademarks of Molecular Probes, Inc.

CF™ is a trademark of Biotium, Inc.



23-9582-07