

BD Horizon Brilliant™ Violet Imaging Reagents

Features

Two very bright dyes, BV421 and BV480, expand color choices for multicolor imaging

Unique spectra allow for easier multiplex staining

Greater sensitivity and resolution in comparison to traditional fluorescent dyes

Photostable

Compatible with most epifluorescence and confocal microscopes

The BD Horizon Brilliant™ polymer dyes were developed exclusively by BD Biosciences. These dyes deliver brighter immunofluorescent reagents compared to conventional dyes with equivalent background—a breakthrough in the field that enables increased sensitivity.

BD Biosciences has optimized two Brilliant Violet dyes, BD Horizon Brilliant™ Violet 421 (BV421) and BD Horizon Brilliant™ Violet 480 (BV480), for imaging applications. The unique spectra of these dyes allow for easier multiplexing. BV421 and BV480 can be used together and in combination with most common imaging dyes, including Alexa Fluor® 488, Alexa Fluor® 555, Alexa Fluor® 594, Alexa Fluor® 647, DRAQ5™, and DRAQ7™. Both dyes are also compatible with eGFP and RFP to better enable immunofluorescent staining with fluorescent protein applications (Figures 1 and 2).

The brightness of BV421 and BV480 provides a high level of signal, enabling better sensitivity and shorter exposure times than standard imaging fluorophores (such as Alexa Fluor® 488) (Figure 3).

BV421 and BV480 are also compatible with standard fixation and permeabilization buffers, as well as common slide-mounting media, such as ProLong® Gold antifade, which results in excellent photostability (Figure 4).



BD Horizon BV421

BV421 is commonly used in flow cytometry but is also an ideal dye for imaging applications. Similar to BV421, BV480 provides a very bright signal, resulting in excellent signal-to-noise resolution. BV421 is detected in the same channel as DAPI. Therefore, when using BV421, either DRAQ5 or DRAQ7 can be used as an alternate nuclear counter-stain.

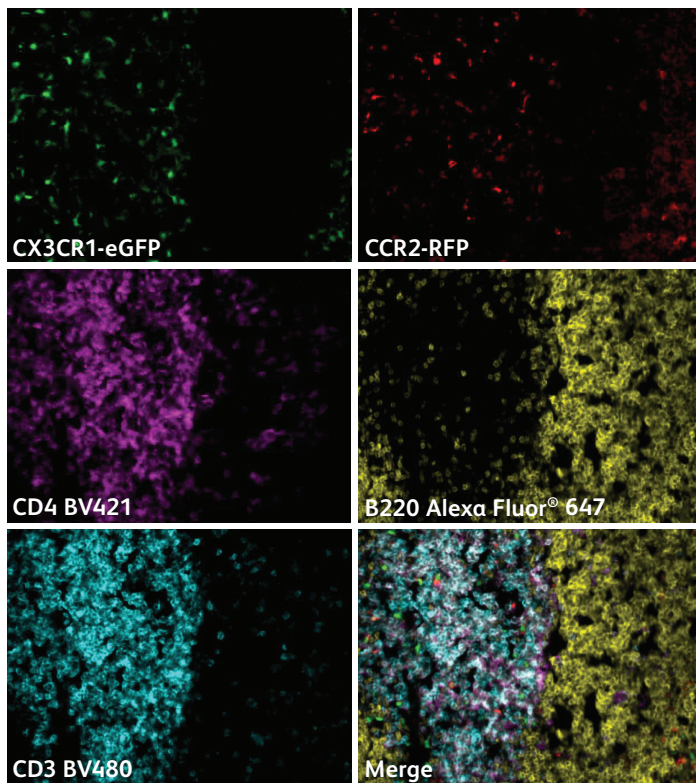


Figure 1. Five-color multiplexed immunohistochemistry

Tissue: Frozen spleen sections from genetic mouse model, CCR2-RFP & CX3CR1-eGFP. **Fix:** BD Cytotfix™ fixation buffer (Cat. No. 554655). **Wash/Perm:** 0.1% Triton™ X-100. **Block/Stain:** 5% goat serum, 1% BSA, 0.5% Triton X-100 in 1X PBS. **Antibodies:** BD Horizon™ BV421 Rat Anti-Mouse CD4 (Cat. No. 562891), BD Horizon™ BV480 Rat Anti-Mouse CD3 (Cat. No. 565642), BD Pharmingen™ Alexa Fluor® 647 Rat Anti-Mouse B220 (Cat. No. 557683). **Other reagents:** ProLong® Gold antifade. Data generated in collaboration with Dr. Catherine Hedrick, La Jolla Institute for Allergy and Immunology.

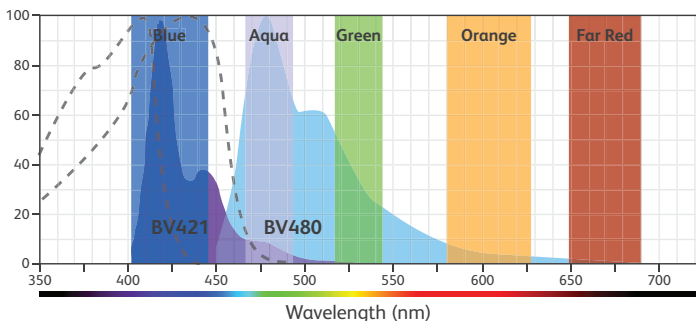


Figure 2. Excitation (dotted lines) and emission (filled histograms) spectra for BV421 and BV480, overlaid with the recommended channels for 5-color imaging

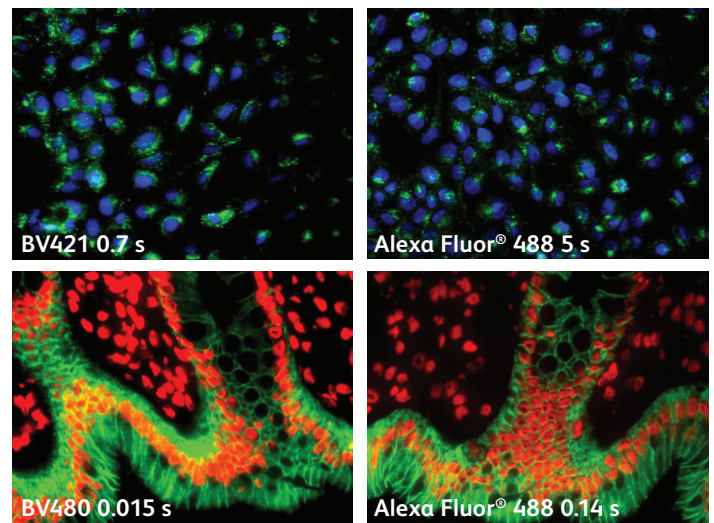


Figure 3. BV421 and BV480 are bright fluorophores

Top: Immunocytochemistry using BV421 or Alexa Fluor® 647 to detect LAMP1 staining in U-2 OS cell line. **Bottom:** Immunohistochemistry using BV480 or Alexa Fluor® 488 to detect E-Cadherin staining on human colon sections. **Antibodies:** BD Horizon BV421 Anti-LAMP1 (Cat. No. 562623) or BD Pharmingen™ Alexa Fluor® 647 Anti-LAMP1 (Cat. No. 562622), BD Transduction Laboratories™ Purified Mouse Anti-E-Cadherin (Cat. No. 610182) with BD Horizon BV480 Goat Anti-Mouse (Cat. No. 564877) or Alexa Fluor® 488 Goat Anti-Mouse (Life Technologies). **Other reagents:** DRAQ5 (Cat. No. 564902), ProLong® Gold antifade. **Instrument:** Acquired on a BD Pathway™ 435 cell analyzer and merged using BD Attovision™ software. 20X objective.

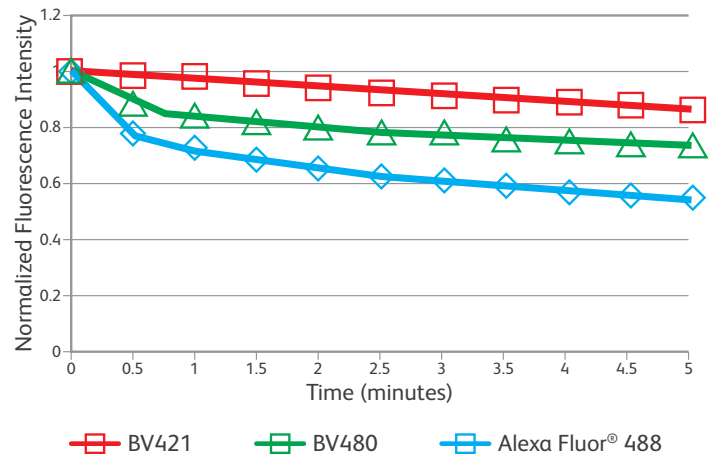


Figure 4. Photostability of BV dyes: continuous exposure to excitation light

Cells: MCF7 cells. **Fixation:** BD Cytotfix fixation buffer. **Antibodies:** BD Pharmingen™ Purified Mouse Anti-Human CD324 (Cat. No. 562869) with BD Horizon BV480 Goat Anti-Mouse (Cat. No. 564877), BD Horizon BV421 Goat Anti-Mouse (Cat. No. 563846) or Alexa Fluor® 488 Goat Anti-Mouse. **Other reagents:** ProLong® Gold antifade. **Instrument:** ImageXpress® Micro XLS (Molecular Devices). 20X objective.

BD Horizon BV480

BD Biosciences has exclusively developed BV480 reagents to improve imaging capabilities. Using BV480 along with Alexa Fluor® 488, Alexa Fluor® 555, Alexa Fluor® 647, and DAPI expands the number of parameters that can be analyzed simultaneously in an imaging application. The exceptional brightness of these reagents provides better signal resolution, and their unique spectra allow for easy multiplexing when used in combination with the appropriate filter configuration (Figure 1, Figure 5 and Table 1).

Table 1. Example filters

Channel (Dye)	Excitation Filter (nm)	Dichroic Mirror (nm)	Emission Filter (nm)
Blue (BV421/DAPI)	392/23	409	430/24
Aqua (BV480)	438/24	458	485/20
Green (Alexa Fluor® 488/eGFP)	485/20	506	537/26
Orange (Alexa Fluor® 555/RFP)	543/22	562	593/40
Far Red (Alexa Fluor® 647/DRAQ5)	628/40	660	692/40

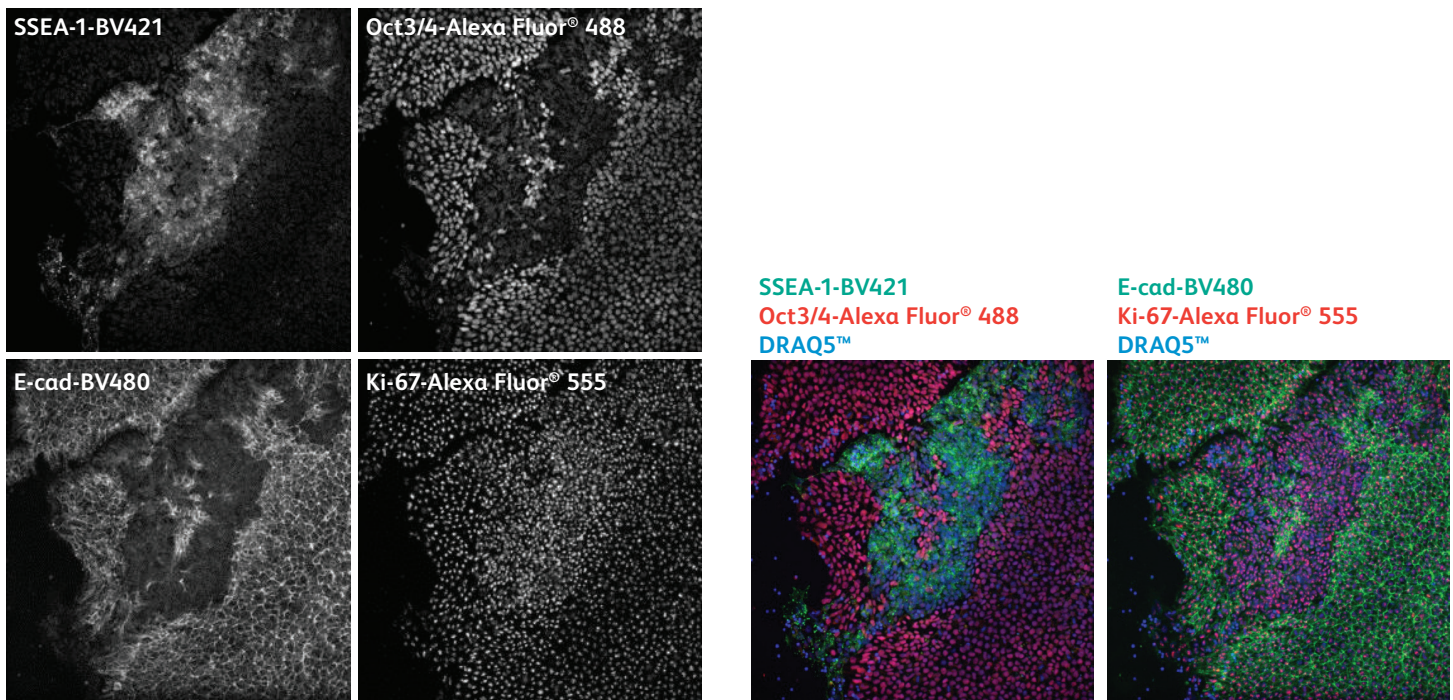


Figure 5. Five-color multiplexed immunocytochemistry

Cells: Human embryonic stem cells (H9) (WiCell). **Fix:** BD Cytofix fixation buffer. **Wash/Perm:** 0.1% Triton X-100. **Block/Stain:** 5% goat serum, 1% BSA, 0.5% Triton X-100 in 1X PBS. **Antibodies:** BD Horizon BV421 Mouse Anti-SSEA-1 (Cat. No. 562705), BD Horizon BV480 Mouse Anti-CD324 (E-Cadherin) (Cat. No. 565646), BD Pharmingen™ Alexa Fluor® 488 Mouse Anti-Oct3/4 (Cat. No. 561628), BD Pharmingen™ Alexa Fluor® 555 Mouse Anti-Human Ki-67 (Cat. No. 558617). **Other reagents:** DRAQ5 (Cat. No. 564902), ProLong® Gold antifade. **Instrument:** Acquired on a BD Pathway 435 cell analyzer and merged using BD Attovision software. 20X objective.

BD Horizon BV421 and BV480 Reagents

Visit bdbiosciences.com for a complete list of products.

Ordering information

Description	Clone	BV480	BV421
Goat anti-Mouse Ig	Polyclonal	564877	563846
Goat anti-Rat Ig	Polyclonal	564878	565013
Goat anti-Rabbit IgG	Polyclonal	564879	565014
Streptavidin	–	564876	563259

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