

Brilliant Violet™ 421 Reagents

Features

Violet-excitable dye with PE-level brightness

Provides excellent population resolution, especially for dim populations

Maximizes choice and flexibility for multicolor panel design

Developed in collaboration with Sirigen, from Nobel Prize winning chemistry

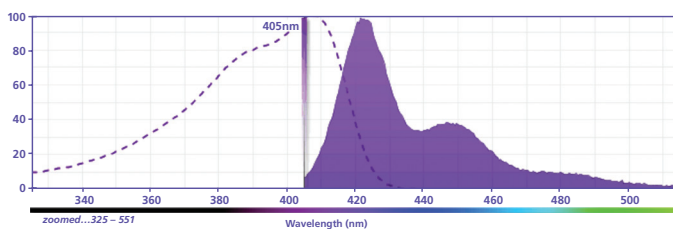


Figure 1. Absorption and emission spectra: Ex Max: 407 nm, Em Max: 421 nm and 448 nm.

| Reagent | Clone | Fluorochrome | Stain Index |
|---------|------------|----------------------|-------------|
| CD4 | RPA-T4 | Brilliant Violet 421 | 561 |
| | | Pacific Blue™ | 24 |
| | | PE | 142 |
| CD8 | RPA-T8 | Brilliant Violet 421 | 782 |
| | | Pacific Blue™ | 29 |
| | | PE | 198 |
| CD127 | hIL-7R-M21 | Brilliant Violet 421 | 55 |
| | | PE | 14 |

Table 1. Lysed whole blood from a single donor stained with CD4, CD8, or CD127 conjugated to Brilliant Violet 421, PE, or Pacific Blue™, run on a BD LSRFortessa™ flow cytometer. All conjugates were run at optimal concentration. Data shown was gated on lymphocytes.

Brilliant Violet™ 421 is a violet-excitable dye with significantly improved brightness over other dyes offered for the violet laser. In addition, these reagents are often brighter than reagents for the blue and red lasers, making Brilliant Violet 421 one of the brightest dyes offered by BD. The brightness of the dye makes it particularly useful in multicolor applications where it can be used to better resolve dim populations.

A bright dye for the violet laser

Brilliant Violet 421 is a polymer-based dye developed by Sirigen from Nobel Prize winning chemistry that provides the high sensitivity fluorescence needed to resolve dim populations. This polymer technology combines the ease of use of a traditional organic dye with the brightness of phycoerythrin (PE) for the violet laser. The Brilliant Violet 421 conjugates are, on average, 10 times brighter than Pacific Blue™ conjugates and are often 2–3 times brighter than PE conjugates (Table 1, Figure 3). Brilliant Violet 421 is optimized for use on BD FACST™ brand flow cytometers equipped with a violet laser, including the BD FACSVers™, the BD™ LSR, the BD FACSAria™, and the BD FACSCanto™ flow cytometer systems. With maximum emission peaks at 421 nm and 448 nm, Brilliant Violet 421 is compatible with the standard BD Horizon™ V450 filter set (eg, 450/50 nm) (Figure 1).

Excellent population resolution

In many cases, the typical violet excitable dyes are not bright enough to adequately resolve dim populations when compared to existing bright fluorochromes such as PE or Alexa Fluor® 647. However, as shown in Table 1, the Brilliant Violet 421 polymer is a very bright fluorochrome that provides excellent population resolution when coupled to antibodies directed at low antigen density markers. This fluorochrome brightness, in conjunction with the low spillover of other fluors into it, contributes to the Brilliant Violet 421 conjugates providing equal or superior resolution over dyes excited by the blue and red lasers.

The example on the following page compares the staining of CD25 and CD127 labeled with Brilliant Violet 421 to that of PE and Alexa Fluor® 647 respectively, in a 7-color panel used to identify regulatory T cells. Two different panels were run in parallel: one contained CD25 Brilliant Violet 421 and CD127 Alexa Fluor® 647 (Figures 2C and 2D) and the other contained CD25 PE and CD127 Brilliant Violet 421 (Figures 2A and 2B). The dot plots demonstrate how using CD25 Brilliant Violet 421 resulted in complete separation between the CD25 negative and positive populations compared to the PE conjugate. The brightness of these two Brilliant Violet 421 conjugates allows for easy and unequivocal identification of the CD25^{bright}CD127^{dim} population (regulatory T cells) as shown in Figures 2B and 2D.

Visit bdbiosciences.com/colors for more information.

Brilliant Violet™ 421 Reagents

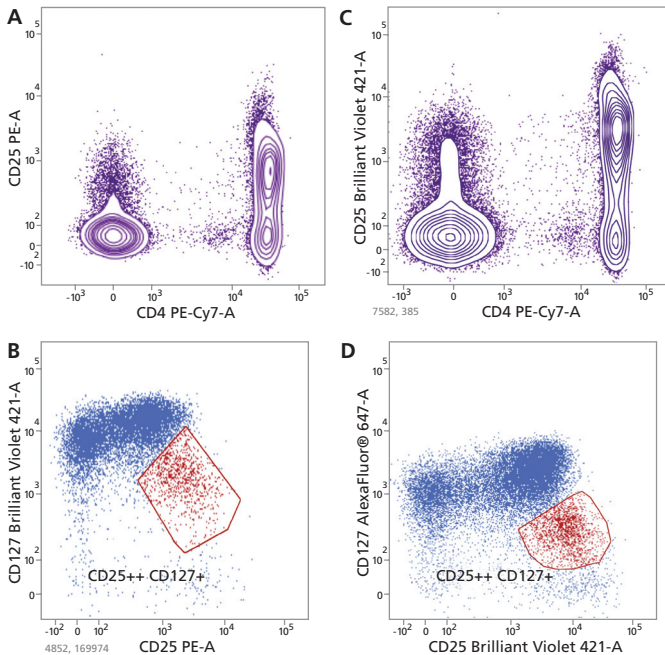


Figure 2. A, B. Lysed whole blood stained with CD3 BD Horizon™ V500, CD4 PE-Cy7™, CD8 PerCP-Cy5.5, CD45RA APC, HLA-DR APC-H7, CD25 PE, and CD127 Brilliant Violet 421. C, D. Lysed whole blood stained with CD3 BD Horizon™ V500, CD4 PE-Cy7, CD8 PerCP-Cy5.5, CD45RA PE, HLA-DR APC-H7, CD127 Alexa Fluor® 647, and CD25 Brilliant Violet 421. The events in Figures 2A and 2C were gated on CD3⁺ lymphocytes. The events in Figures 2B and 2D were gated on CD3⁺CD4⁺ lymphocytes. All data was run on a BD FACSVerser flow cytometer.

Brilliant Violet 421 RUO Reagents

| Description | React. | Clone | Isotype | Size | Cat.No. |
|-------------|--------|--------------|-------------------------|-----------|---------|
| CD3 | Hu | UCHT1 | Ms IgG ₁ , κ | 25 tests | 562427 |
| | | | | 100 tests | 562426 |
| CD4 | Hu | RPA-T4 | Ms IgG ₁ , κ | 25 tests | 562425 |
| | | | | 100 tests | 562424 |
| CD8 | Hu | RPA-T8 | Ms IgG ₁ , κ | 25 tests | 562429 |
| | | | | 100 tests | 562428 |
| CD19 | Hu | HIB19 | Ms IgG ₁ , κ | 25 tests | 562441 |
| | | | | 100 tests | 562440 |
| CD25 | Hu | M-A251 | Ms IgG ₁ , κ | 25 tests | 562443 |
| | | | | 100 tests | 562442 |
| CD27 | Hu | M-T271 | Ms IgG ₁ , κ | 25 tests | 562514 |
| | | | | 100 tests | 562513 |
| CD38 | Hu | HIT2 | Ms IgG ₁ , κ | 25 tests | 562445 |
| | | | | 100 tests | 562444 |
| CD73 | Hu | AD2 | Ms IgG ₁ , κ | 25 tests | 562431 |
| | | | | 100 tests | 562430 |
| CD86 | Hu | 2331 (FUN-1) | Ms IgG ₁ , κ | 25 tests | 562433 |
| | | | | 100 tests | 562432 |
| CD117 | Hu | YB5.B8 | Ms IgG ₁ , κ | 25 tests | 562435 |
| | | | | 100 tests | 562434 |
| CD123 | Hu | 9F5 | Ms IgG ₁ , κ | 50 tests | 562517 |

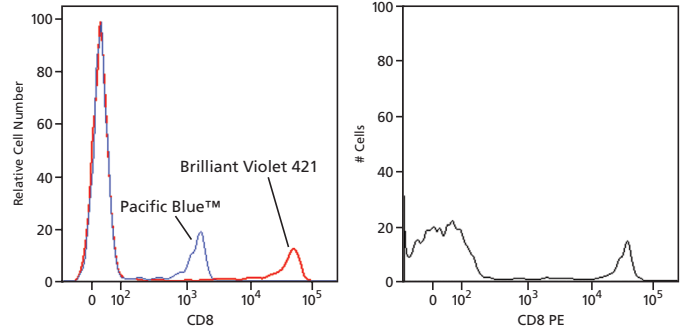


Figure 3. Lysed whole blood stained with CD8 Brilliant Violet 421 or Pacific Blue™ (left), and PE (right).

Compatible with standard surface and intracellular staining protocols

Brilliant Violet 421 is compatible with standard buffers used in surface and intracellular staining protocols, making the reagents easy to incorporate into current workflows. These reagents also demonstrate compatibility in paraformaldehyde-based fixatives and both EDTA and heparin blood collection tubes.

Tools to optimize setup, selection, and performance

To help advance the use of multicolor flow cytometry, BD Biosciences offers a growing library of tools and resources relevant to both experienced researchers and those new to multicolor panel design (bdbiosciences.com/colors). In addition to this online resource, BD Biosciences offers one-on-one technical application support as part of our comprehensive customer services.

| Description | React. | Clone | Isotype | Size | Cat.No. |
|---------------------------|--------|------------|---------------------------|-----------|---------|
| CD127 | Hu | HIL-7R-M21 | Ms IgG ₁ , κ | 25 tests | 562437 |
| | | | | 100 tests | 562436 |
| CD184 | Hu | 12G5 | Ms IgG _{2a} , κ | 50 tests | 562448 |
| CD196 | Hu | 11A9 | Ms IgG ₁ , κ | 50 tests | 562515 |
| CD279 | Hu | EH12.1 | Ms IgG ₁ , κ | 50 tests | 562516 |
| CD3e | Ms | 145-2C11 | Ham IgG ₁ , κ | 50 µg | 562600 |
| CD11b | Ms | M1/70 | Rat IgG _{2b} , κ | 50 µg | 562605 |
| CD25 | Ms | PC61 | Rat IgG ₁ , λ | 50 µg | 562606 |
| CD34 | Ms | RAM34 | Rat IgG _{2a} , κ | 50 µg | 562608 |
| CD117 | Ms | 2B8 | Rat IgG _{2b} , κ | 50 µg | 562609 |
| CD138 | Ms | 281-2 | Rat IgG _{2a} , κ | 50 µg | 562610 |
| Ham IgG ₁ , κ | - | A19-3 | - | 50 µg | 562601 |
| Ms IgG ₁ , κ | - | X40 | - | 50 µg | 562438 |
| Ms IgG _{2a} , κ | - | G155-178 | - | 50 µg | 562439 |
| Rat IgG _{2a} , κ | - | R35-95 | - | 50 µg | 562602 |
| Rat IgG _{2b} , κ | - | R35-38 | - | 50 µg | 562603 |
| Rat IgG ₁ , λ | - | A110-1 | - | 50 µg | 562604 |



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