Brilliant Violet 421™ antibody conjugates deliver consistent and superior performance on violet-laser equipped flow cytometers with varied specifications

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Abstract

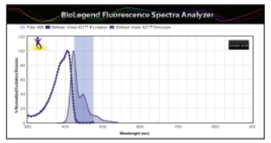
Brilliant Violet 421™ antibody conjugates deliver consistent and superior performance on violet-laser equipped flow cytometers with varied specifications

Brilliant Violet™ derived fluorophores are novel and highly sensitive polymeric molecules that fluoresce upon excitation by a 405 nm laser line. In this study, Brilliant Violet 421™- antibody conjugates were evaluated for their performance on violet laser equipped flow cytometers with varying instrument configurations. Instrument specific variables included sheath pressure, laser power, PMT gain and position, optical configuration, and filter sets. Cross beam spectral overlap was also evaluated for 488nm and 561nm lasers. All instrument configurations revealed significantly increased signal to noise ratio with Brilliant Violet™ as compared to traditional violet laser dyes. BV421™ yielded logarithmic improvements in signal to noise ratio, while maintaining manageable spectral compensation properties. BV421™ antibody conjugates against high and low-density surface markers also increased resolution and accuracy, notably for human CD25, CD56, CD127 and mouse NK1.1 and CD4. The Brilliant Violet™ conjugates were also found to be effective for detection of intracellular cytokines. Overall, Brilliant Violet 421™ and were determined to outperform traditional violet-excitable organic dyes on flow cytometers with a variety of specifications and settings.

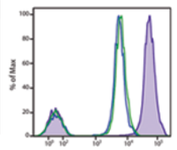
Introduction

In building multicolor flow cytometry panels, researchers are presented with challenges optimizing myriad parameters. Considerations include the scientific question to be addressed and the compatibility of the flow cytometer to analyze mulitiple parameters. Once a panel of antigens has been selected for assay, a savvy investigator pairs these antigens with fluorophores to report robust and accurate data with a primary aim of pairing readily detectable, bright fluorophores with dimly expressed antigens. Limited fluorophore availability often precedes the compromised pairing of a moderately expressed antigen with a less than bright fluorophore. The 405nm violet laser-excitable dye, Brilliant Violet 421™, was introduced to expand the choice of fluorophores for both single and multicolor flow cytometry. To validate this application, BV421™ was evaluated for its ability to enhance detection of dimly expressed antigens. Further, the spectral properties of BV421™ antibody conjugates were described using flow cytometers of varied design and configuration.

Figure 1. BV421™ is brighter than Pacific Blue™ and BD Horizon™ V450 and detectable with the same filter set



http://www.biolegend.com/spectraanalyzer



Stain Index			
Specificity /Clone	Fluorochrome	Laser Excitation	Stain Index
CD3/UCHT1	Brilliant Violet 421™	405 nm	262
CD3/UCHT1	PE	561 nm	170
CD3/UCHT1	Pacific Blue™	405 nm	59
CD3/UCHT1	BD Horizon™ V450	405 nm	56

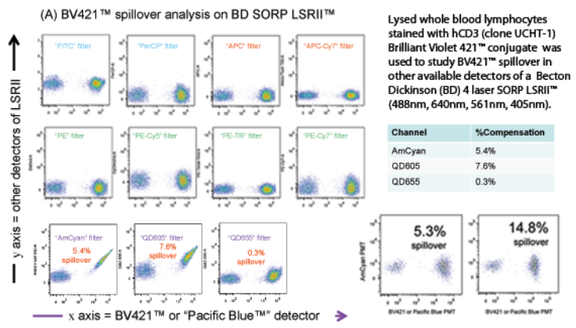
Lysed whole blood lymphocytes stained with hCD3 (UCHT-1) conjugates was used to measure Stain Index (4-laser BD SORP LSRII™)

Conclusion

Successful multicolor cellular analysis relies on the performance of fluorochromes. Our studies have demonstrated that Brilliant Violet™ 421:

- Can be run on flow cytometers equipped with violet lasers
- Is excited by a wide range of violet laser power settings
- Exhibits low cross-beam excitation promiscuity
- Resists degradation by common fixation reagents
- Requires less compensation than Pacific Blue™ for spillover into AmCyan, QD605 and QD655 channels
- Enhances detection of low density antigens
- Is significantly brighter than Pacific Blue™ and other similar dyes
- Is compatible with intracellular staining techniques (e.g., cytokine detection)
- Enables enhanced detection of low frequency populations when using multicolor panels
- Performed equivalently on both cuvette or jet-in-air design high speed cell sorters (data not shown)
- Suitable for microscopy with minimal photobleaching compared to other fluorochromes (data not shown)

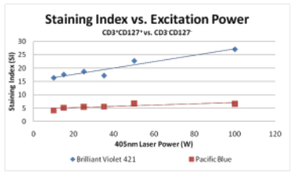
Figure 2. Brilliant Violet 421™ has low spillover into other detectors



Lysed whole blood lymphocytes stained with hCD3 (clone UCHT-1) Brilliant Violet 421™ conjugate was used to study BV421™ spillover in other available detectors of a Becton Dickinson (BD) 4 laser SORP LSRII™ (488nm, 640nm, 561nm, 405nm).

Channel	%Compensation
AmCyan	5.4%
QD605	7.6%
QD655	0.3%

Figure 3. Increased laser power enhances staining index of Brilliant Violet 421™ and Pacific Blue™



100mW Violet laser

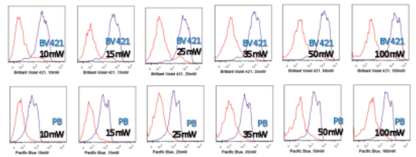
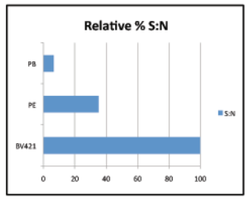


Figure 3: Comparison of Staining Index of BV421™ and Pacific Blue™. Stain Index was calculated for these populations by:

$$(SI) = \frac{\chi CD3^{low}CD127^{pos} - \chi CD3^{low}CD127^{neg}}{(2 \times SDCD3^{low}CD127^{neg})}$$

Human peripheral blood lymphocytes were stained with CD3-APC and CD127 Brilliant Violet 421™ or Pacific Blue™, then interrogated by flow cytometry using a tunable wattage 405nm laser. Laser power was adjusted to intervals of 10mW, 15mW, 25mW, 35mW, 50mW and 100mW. Doublets were excluded and histogram overlays were generated for CD3-CD127- (red) and CD3+CD127+ (purple) cells signal as measured through the 450/50 band pass filter.

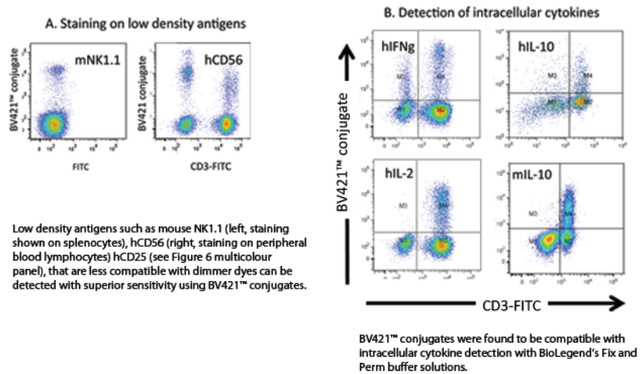
Figure 4. Use of Brilliant Violet 421™ conjugated antibodies results in increased signal to noise



Clone	Specificity	BV421	PE	PB
BC96	hCD25	100	8	1
BM8	hF4/80	100	53	18
EH12.2H7	hCD279	100	18	6
FN50	hCD69	100	91	15
HCD56	hCD56	100	15	3
HI100	hCD45RA	100	20	3
RPA-T8	hCD8	100	23	2
UCHT1	hCD3	100	45	7
145-2C11	mCD3	100	45	2
GK1.5	mCD4	100	57	7
N418	mCD11c	100	27	10
PK136	mNK1.1	100	24	4
Avg			35.5	6.5

Conjugates of BV421™, phycoerythrin (PE) and Pacific Blue™ (PB) were directly compared in the same staining assay. The signal:noise ratio (S:N) of the positive target cells obtained with the PE and PB conjugates was expressed as a percentage of the S:N obtained with the BV421™ conjugate. Results for 12 clones revealed that, on average, the S:N was 29% and 4% of the BV421™ S:N for PE and PB respectively.

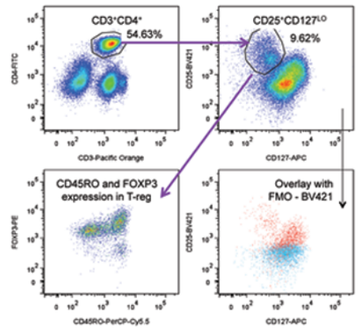
Figure 5. Brilliant Violet 421™ conjugates for dim antigen detection and intracellular staining



Low density antigens such as mouse NK1.1 (left, staining shown on splenocytes), hCD56 (right, staining on peripheral blood lymphocytes) hCD25 (see Figure 6 multicolour panel), that are less compatible with dimmer dyes can be detected with superior sensitivity using BV421™ conjugates.

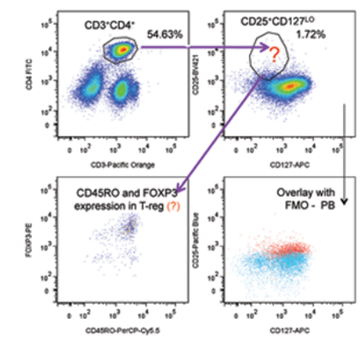
BV421™ conjugates were found to be compatible with intracellular cytokine detection with BioLegend's Fix and Perm buffer solutions.

Figure 6. Brilliant Violet 421™ for multi-colour detection of low density antigens, is tolerant of fixation and permeabilization, providing a powerful new option for flow cytometry



T reg panel with CD25-BV421™

Antigens	Fluor
CD3	Pacific Orange™
CD4	FITC
CD127	APC
CD25	BV421™ or PB
FOXP3	PE
CD45RO	PerCP-Cy5.5



T reg panel with CD25-Pacific Blue™

Human peripheral blood lymphocytes were stained for surface markers (Left Table) then fixed and permeabilized before staining for FOXP3.

FOXP3 Staining: BioLegend's FOXP3 fix/perm buffer set and FOXP3 staining protocol

FOXP3-PE antibody (clone 206D)

Learn more about Brilliant Violet™ antibodies at www.biolegend.com/brilliantviolet.