

Brilliant Violet™

Antibody Conjugates

**Complete
Brilliance**
for the Violet Laser

Novel Fluorophore
Conjugates for
Flow Cytometry



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02-0005-01

World-Class Quality | Superior Customer Support | Outstanding Value

Introduction

Brilliant Violet™ antibody conjugates, proudly co-developed by BioLegend and Sirigen, are an innovative class of novel research reagents, providing more options for multicolor flow cytometry panels and superior results.

Brilliant Violet™ Technology

The Brilliant Violet™ family of fluorescent molecules are organic polymers with an extraordinary capacity to absorb energy (extinction coefficient) and a high efficiency with which to convert that absorbed energy to an emitted signal (quantum yield). When conjugated to antibodies, this results in high intensity brightness on labeled cells.

Physical Properties

Brilliant Violet 421™ has an extinction coefficient of 2,500,000 M⁻¹cm⁻¹ at 405 nm, an aqueous solution quantum yield of 65 ± 5%, and solubility in excess of 50 mg/mL in PBS. The extinction coefficient contributes to its superior brightness compared to Pacific Blue™, which has an extinction coefficient of 30,000 M⁻¹cm⁻¹.

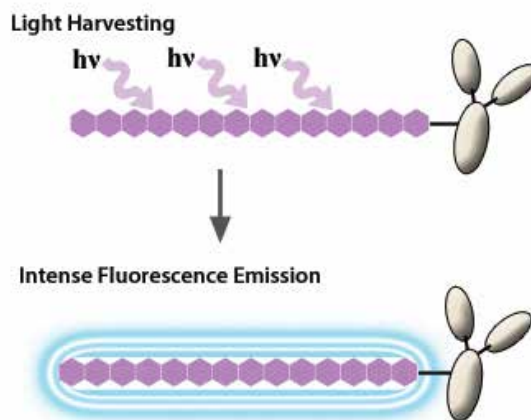
Brilliant Violet 421™ can also be modified with another dye to produce high stokes shift emissions. Brilliant Violet 570™, Brilliant Violet 605™, Brilliant Violet 650™, Brilliant Violet 711™, and Brilliant Violet 785™ are such derivatives of the Brilliant Violet 421™ polymer, emitting maximally at 570 nm, 603 nm, 645 nm, 711 nm, and 785 nm, respectively, when excited at 405 nm.

Brilliant Violet 510™, another High Sensitivity Fluorescence™ polymer, is the newest violet laser excitable fluorophore with maximal emission at 510 nm.

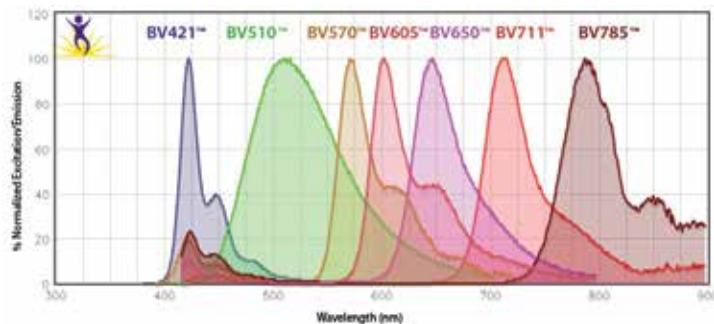
This figure provides the emission spectra of the Brilliant Violet™ fluorophores. To view excitation data and to compare with other fluorophores, use our Spectra Analyzer tool at: [biolegend.com/spectraanalyzer](https://www.biolegend.com/spectraanalyzer).

Easy to Use and Trouble-Free

Brilliant Violet™ antibody conjugates are simple to use, compatible with standard staining buffers, and stable to fixation. Provided in convenient 5 µl test sizes at optimal ready-to-use concentrations, our Brilliant Violet™ antibody products can easily be added to your multicolor panels. We are now introducing µg sizes for optimal value.



Emission Spectra of Brilliant Violet™ Fluorophores



For more information, visit: [biolegend.com/brilliantviolet](https://www.biolegend.com/brilliantviolet)

Brilliant Violet™ Fluorophore Family at a Glance

Brilliant Violet 421™	Brilliant Violet 510™	Brilliant Violet 570™
Excitation max = 405 nm	Excitation max = 405 nm	Excitation max = 405 nm
Emission max = 421 nm	Emission max = 510 nm	Emission max = 570 nm
Recommended filter = 450/50	Recommended filter = 510/50	Recommended filter = 585/42
Comparable Fluorophores Pacific Blue™ BD Horizon™ V450 Alexa Fluor® 405 Cascade Blue™ eFluor® 450	Comparable Fluorophores BD Horizon™ V500 AmCyan	Comparable Fluorophores Pacific Orange™ Qdot® 565 Cascade Yellow™ eFluor® 565NC Qdot® 545 Krome Orange™
Brightness = 5 On a scale from 1 to 5, with 5 being the brightest. Molar Extinction Coeff.= 2,500,000 M ⁻¹ cm ⁻¹ Quantum Yield = 0.65 in DPBS	Brightness = 3 Molar Extinction Coeff.= 577,000 M ⁻¹ cm ⁻¹ Quantum Yield = 0.44 in DPBS	Brightness = 2-3 Molar Extinction Coeff.= 2,300,000 M ⁻¹ cm ⁻¹ Quantum Yield = 0.08 in DPBS
MW = 60- 80 kD	MW = 60- 80 kD	MW = 60- 80 kD

Brilliant Violet 605™	Brilliant Violet 650™	Brilliant Violet 711™
Excitation max = 405 nm	Excitation max = 405 nm	Excitation max = 405 nm
Emission max = 603 nm	Emission max = 645 nm	Emission max = 711 nm
Recommended filter = 610/20	Recommended filter = 660/20	Recommended filter = 710/50
Comparable Fluorophores Qdot® 605 eFluor® 605NC	Comparable Fluorophores Qdot® 655 eFluor® 650NC	Comparable Fluorophores eFluor® 700NC Qdot® 705
Brightness = 4 Molar Extinction Coeff.= 2,400,000 M ⁻¹ cm ⁻¹ Quantum Yield = 0.29 in DPBS	Brightness = 3 Molar Extinction Coeff.= 2,500,000 M ⁻¹ cm ⁻¹ Quantum Yield = 0.17 in DPBS	Brightness = 3 Molar Extinction Coeff.= 2,800,000 M ⁻¹ cm ⁻¹ Quantum Yield = 0.15 in DPBS
MW = 60- 80 kD	MW = 60- 80 kD	MW = 60- 80 kD

Brilliant Violet 785™
Excitation max = 405 nm
Emission max = 785 nm
Recommended filter = 780/60
Comparable Fluorophores Qdot® 800
Brightness = 3 Molar Extinction Coeff.= 2,500,000 M ⁻¹ cm ⁻¹ Quantum Yield = 0.04 in DPBS
MW = 60- 80 kD

Brilliant Violet™ Considerations for Multicolor Flow Cytometry

Brilliant Violet 421™ (BV421™)



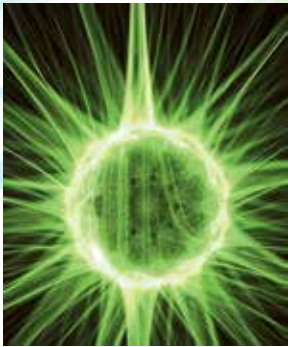
- BV421™ has an emission spectrum that is more narrow than that of Pacific Blue™; thus, there is less spillover into neighboring channels, such as AmCyan or Horizon™ V500.
- BV421™ is excitable to some degree by the UV laser (350-355 nm) (~25% of maximal excitation), so some compensation is required when used in combination with UV-excited fluorophores. However, BV421™ is consistently demonstrated to be compatible with viability probes Fixable Live/Dead Blue or DAPI excited off the UV laser. BV421™ is not excited by other lasers, such as the 488, 532 or 561 nm.
- For most cell surface staining, BV421™ is significantly brighter than equivalent fluorophores, including Pacific Blue™, BD Horizon™ V450, eFluor® 450, and Alexa Fluor® 405. It has an extinction coefficient of 2,500,000 M⁻¹cm⁻¹ at 405 nm, and an aqueous solution quantum yield of 65 ± 5%. On a brightness scale of 1 - 5, with 5 being the brightest, we give this a 5.
- It may be possible to use BV421™ together with Pacific Blue™, but very specific and narrow bandpass filters would be needed. BioLegend does not recommend attempting this for the average flow user, as compensation requirements will likely be very high. In addition, stray light from the violet laser can increase the background signal for BV421™.

Brilliant Violet 510™ (BV510™)



- BV510™ has a similar emission spectrum to that of Horizon™ V500, so we recommend using the bandpass filter setting being used for Horizon™ V500 or Live/Dead Aqua (510/50 or 525/50).
- BV510™ is excitable by the UV laser (350-355 nm), so some compensation is required when used in combination with UV-excited fluorophores. BV510™ is not excited by other lasers, such as the 488, 532 or 561 nm.
- For cell surface staining, BV510™ is significantly brighter than equivalent fluorophores, including Pacific Orange™ and BD Horizon™ V500. On a brightness scale of 1 - 5, with 5 being the brightest, we give this a 3.
- When using BV510™ with other Brilliant Violet™ fluorophores, compensation and PMT voltage balancing with BV570™ and some BV605™ is required. A good strategy when choosing markers for BV510™ and BV570™ is to choose markers that will not be co-expressed on the same cell.

Brilliant Violet 570™ (BV570™)



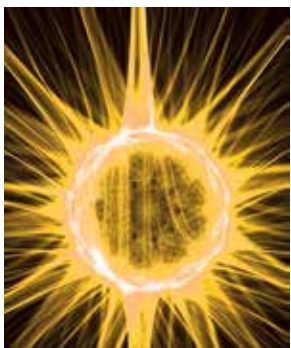
- BV570™ is excited by the violet laser at 405 nm, emits maximally at 570 nm, and can be used in place of Pacific Orange™, Qdot® 565, Qdot® 585, eFluor® 565NC, and eFluor® 585NC.
- BV570™ antibody conjugates provide a good signal-to-noise ratio, although not as bright as BV421™. On a brightness scale of 1-5, with 5 being the brightest, we give this a 2-3.
- The optimal bandpass filter (585/42) is typically not the default filter on most instruments. Be sure that this bandpass filter is correctly configured on the instrument before using BV570™.
- Use the 575LP filter when using BV570™ with Horizon™ V500 or Fixable Live/Dead Aqua, in order to prevent unnecessary spillover and with instruments equipped with 561 nm laser line. In most other cases, using a LP filter between 545 and 556 nm is acceptable.

- BV570™ has an emission spectrum very similar to that of PE and it can be partially excited by the green laser (532 nm), the yellow-green laser (561 nm), and to a lower extent, by the blue laser (488 nm). This raises potential compensation issues when using the two fluorophores together in a multicolor panel. In order to minimize spill-over/compensation requirements for the PE channel, we advise that users adjust the PMT-V for BV570™ to be higher than PMT-V for PE. The data below is an example of % compensation requirements for two PMT-voltage scenarios, one in which the BV570™ PMT-V is higher and one where the PE PMT-V is higher. Note that the % compensation into BV421™ and PE is significantly less when the BV570™ PMT-V is higher.

PMT	BV570™	Voltage
PE	5.70%	520
BV421™	6.00%	536
BV570™	—	550
PE	20.42%	600
BV421™	12.57%	600
BV570™	—	550

*This is only an example for one specific instrument and configuration. Optimization will be required for your specific instrument and configuration. Using a 575LP for the PE detector also helps to reduce the spillover of BV570™ into PE.

Brilliant Violet 605™ (BV605™)



- BV605™ is excited by the violet laser at 405 nm and emits maximally at 603 nm, and can be used in place of Qdot® 605 and eFluor® 605NC.
- BV605™ antibody conjugates provide excellent signal-to-noise ratio with brightness on par with that of BV421™ or PE. On a brightness scale of 1-5, with 5 being the brightest, we give this a 4.

- BV605™ requires very little compensation with other lasers. When used in a panel with BV570™ and BV650™ on the violet laser, PMT voltage balancing is required, which means that the default CS&T settings will likely not be optimal when using these together.
- The standard Qdot® 605 filter for this PMT, 610/20 with a 595LP dichroic, works well for BV605™ detection.

Brilliant Violet™ Considerations for Multicolor Flow Cytometry *(Continued)*

Brilliant Violet 650™ (BV650™)



- BV650™ is excited by the violet laser at 405 nm and emits maximally at 645 nm, and can be used in place of Qdot® 655 and eFluor® 650NC.
- BV650™ antibody conjugates provide excellent signal-to-noise ratio, with brightness rated at 3 on a scale of 1-5, with 5 being the brightest.
- BV650™ has some slight compensation requirements with APC, due to its partial excitation by the 633 nm laser. The compensation requirements are minimal and do not normally require any special adjustments.
- The standard Qdot® 655 filter for this PMT, 660/20 with a 630nm LP dichroic, works well for BV650™ detection. When being used together with BV605™, we recommend the 660/20 or 670/20 BP filter to minimize spillover.

Brilliant Violet 711™ (BV711™)



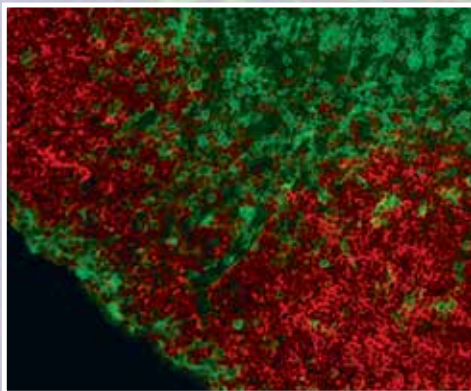
- BV711™ is excited by the violet laser at 405 nm and emits maximally at 711 nm, and can be used in place of Qdot® 705 and eFluor® 700NC.
- BV711™ antibody conjugates provide good brightness, rated at 3 on a scale of 1-5, with 5 being the brightest.
- The standard Qdot® 700 filter for this PMT, 710/50 with a 685nm LP dichroic, works well for BV711™ detection.
- BV711™ has some spillover into 633 nm laser-excited fluorophores, due to its partial excitation by the 633 nm laser, as well as moderate spillover into PerCP/Cy5.5. The compensation requirements are manageable and do not normally require any special adjustments.
- BV711™ will have moderate spillover into BV785™. Compensation requirements may be high, but manageable. When used together, we recommend BV785™ for more highly expressed antigens and BV711™ for lower expressing antigens.

Brilliant Violet 785™ (BV785™)



- BV785™ is excited by the violet laser at 405 nm, emits maximally at 785 nm, and can be used in place of Qdot® 800.
- BV785™ antibody conjugates provide good brightness, rated at 3 on a scale of 1-5, with 5 being the brightest. BV785™ has some cross-beam excitation by the red laser and thus a small amount of spillover into the APC/Cy7 channel.
- The standard Qdot® 800 filter for this PMT, 780/60 with a 750 nm LP dichroic, works well for BV785™ detection.

Brilliant Violet 421™ in Immunofluorescence Microscopy



BL/6 mouse lymph nodes, stained with CD19-BV421™ (red) and CD4-Alexa Fluor® 488 (green). Image provided by Ann Haberman and Christine Podolski, Yale University.



BL/6 mouse lymph nodes, stained with I-A/I-E BV421™ (red), B220 Alexa Fluor® 488 (blue), and CD11c FITC (green). Image provided by Ann Haberman and Christine Podolski, Yale University.

Brilliant Results

Brilliant Violet™ antibody conjugates have been thoroughly tested by our in-house scientific team, as well as by external testers, to ensure that our products are at the highest level of quality. Products have been validated by various labs worldwide, on a variety of instruments, using a wide variety of applications, including flow cytometry and confocal microscopy. Testing not only ensures the usefulness of Brilliant Violet™ products in these applications, but also verifies that the products are easy-to-use, simple to integrate into complex multicolor panels, and effective at producing the best possible results.

Brilliant Violet 421™

High sensitivity for mouse CD127

Cat. No.	Description	Clone
135023/4	Brilliant Violet 421™ anti-mouse CD127 (IL-7Rα)	A7R34

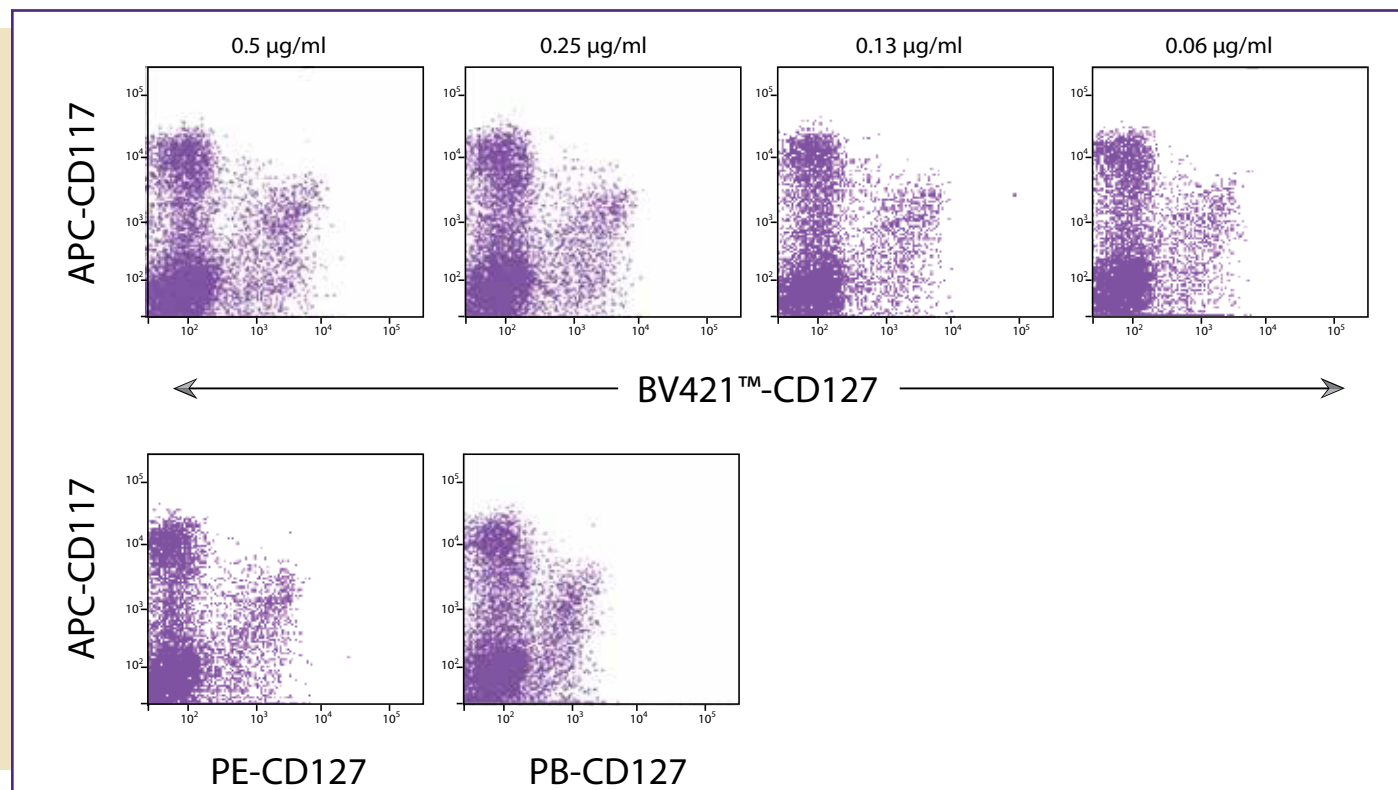
Brilliant Sensitivity

The high intensity brightness of Brilliant Violet 421™ enables visualization of weakly expressed antigens. It can also allow researchers to use higher dilutions of antibody to obtain the same data, resulting in less antibody per test. Here, researchers were able to use dilutions of Brilliant Violet 421™ anti-mouse CD127 to identify precursor cells from bone marrow in a 6-color panel. At

all dilutions tested, the BV421™ outperformed the Pacific Blue™ equivalent used at much higher concentrations (10 µg/mL). The BV421™ results were comparable to those of the PE conjugate. The total amount of antibody was critical in this sorting experiment because the desired population was less than 2% of total stained cells, and the starting amount was 500 million bone marrow cells.



Data Courtesy of Renee De Pooter and David Leclerc, University of Chicago.



Brilliant Violet 421™

Resolution of mouse NK1.1 populations

Cat. No.	Description	Clone
108731/2	Brilliant Violet 421™ anti-mouse NK-1.1	PK136

Brilliant Resolution

Resolution is critical for identifying both rare cell populations and dimly expressed markers. NK1.1 is commonly used for studying NK and NKT cells in the mouse model. Here, researchers demonstrate the resolving power of Brilliant Violet 421™, with brightness on par with PE-conjugated antibody, resulting in similar population percentages. The BV421™ antibody is able to clearly resolve the NK and NKT cell populations, providing far superior signal-to-noise compared to BD Horizon™ V450 antibody.

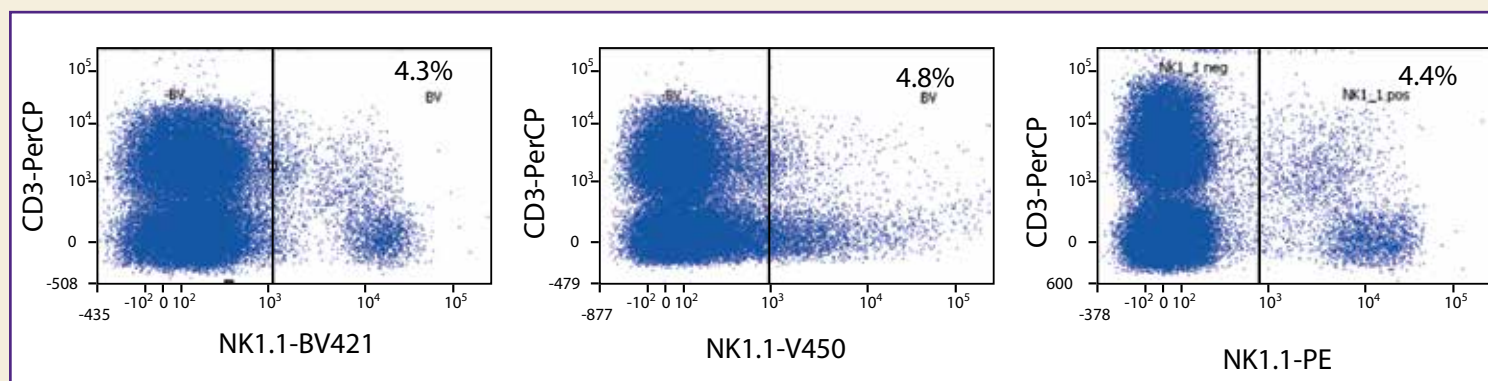
Resolution of human CD45RA vs. CD45RO populations

Cat. No.	Description	Clone
304129/30	Brilliant Violet 421™ anti-human CD45RA	HI100
400341/2	Brilliant Violet 421™ Mouse IgG2b, κ Isotype Ctrl	MPC-11

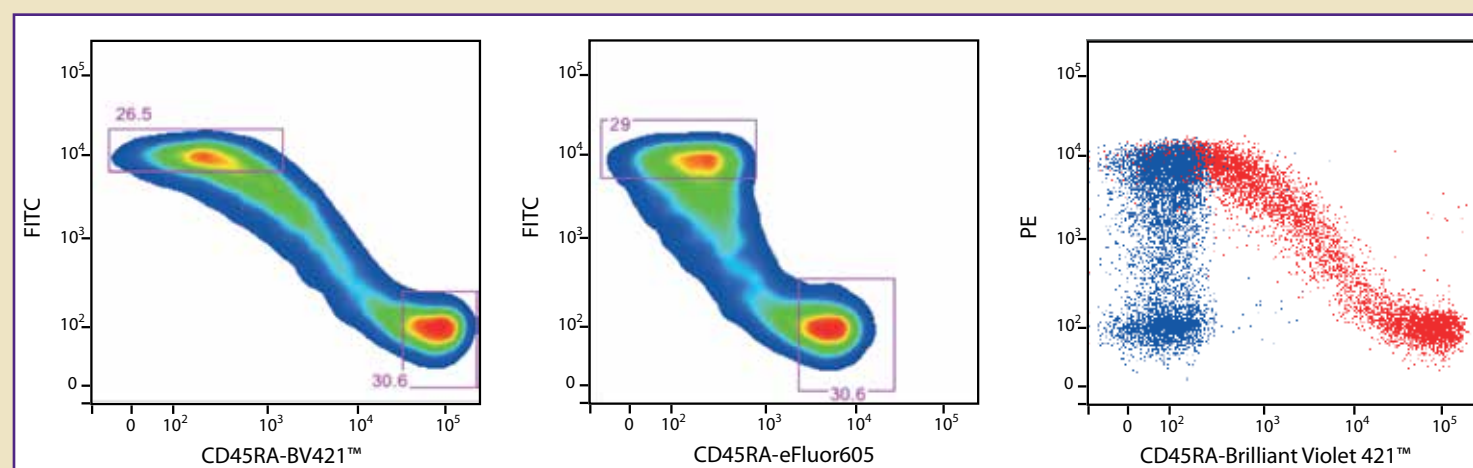
Brilliant Resolution

Resolution is critical for identifying both transitioning cell populations and fully differentiated populations. In T-cell studies, CD45RA is used to identify naïve T cells, while CD45RO identifies activated and memory T cell populations. Here, researchers demonstrate that Brilliant Violet 421™ -conjugated anti-CD45RA gives a significantly improved (one log greater) dynamic range compared to eFluor® 605NC-conjugated antibody (A and B). The BV421™ -antibody conjugate allows for visualization of transitioning populations and clearly distinguishes the fully differentiated memory population from the naïve population. The isotype control at the same concentration provides a very low background, further demonstrating the increased dynamic range of BV421™ antibodies, with highly bright positive populations and low non-specific staining.

Data courtesy of Eva Tolosa and Timo Lischke at UKE Hamburg.



Data Courtesy of Marcin Pekalski and Linda Wicker at Cambridge Institute for Medical Research, Diabetes and Inflammation Lab.



Brilliant Violet 421™

Tetramer Resolution – mouse CD1d

Cat. No.	Description
405225/6	Streptavidin-Brilliant Violet 421™

Brilliant Sensitivity

The high intensity brightness of Brilliant Violet 421™ enables high resolution of rare tetramer-stained populations. Tetramer assays are designed to identify antigen-specific T cell populations through the use of peptide-loaded MHC tetramers labeled with a fluorochrome. Here, researchers compared PBS57-loaded mouse CD1d tetramers labeled with Streptavidin-Brilliant

Violet 421™, PE, or Pacific Blue™. BV421™ outperformed both PE and Pacific Blue™ at equivalent tetramer concentrations. In this scenario, the Pacific Blue™ signal is too weak to provide any resolution of positive populations. BV421™ provided excellent signal-to-noise at all concentrations tested.

Functional cells post-sorting with mouse CD4

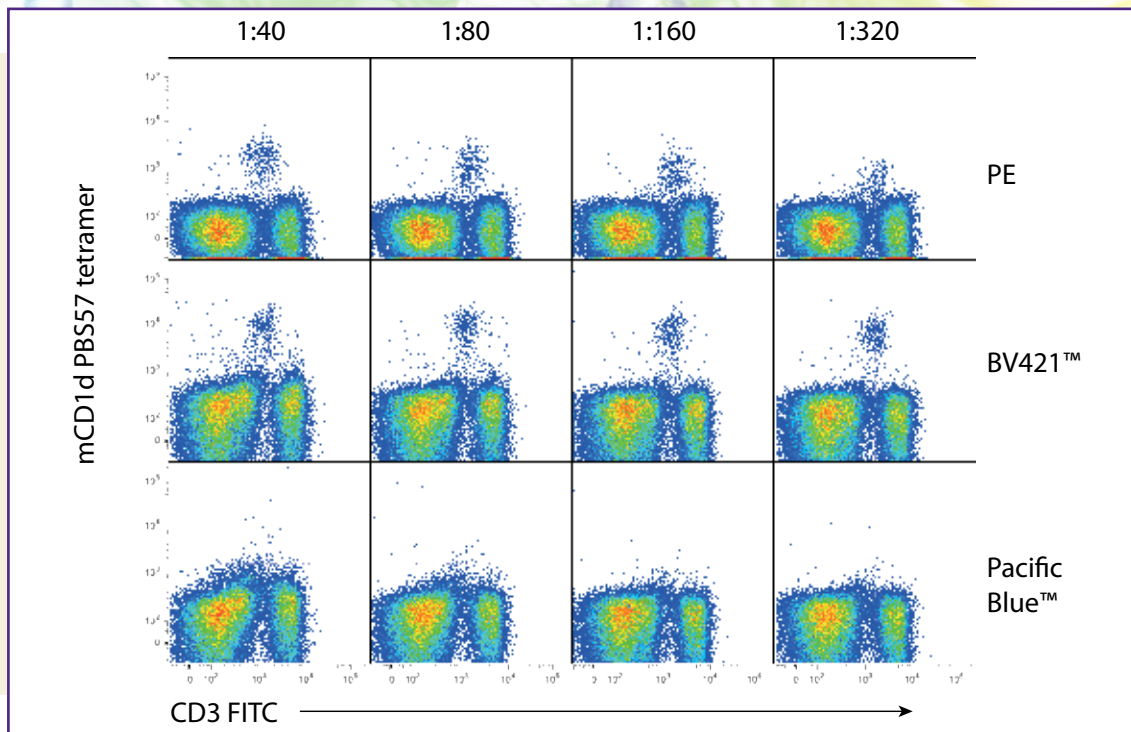
Cat. No.	Description	Clone
100437/8	Brilliant Violet 421™ anti-mouse CD4	GK1.5

Brilliant Viability

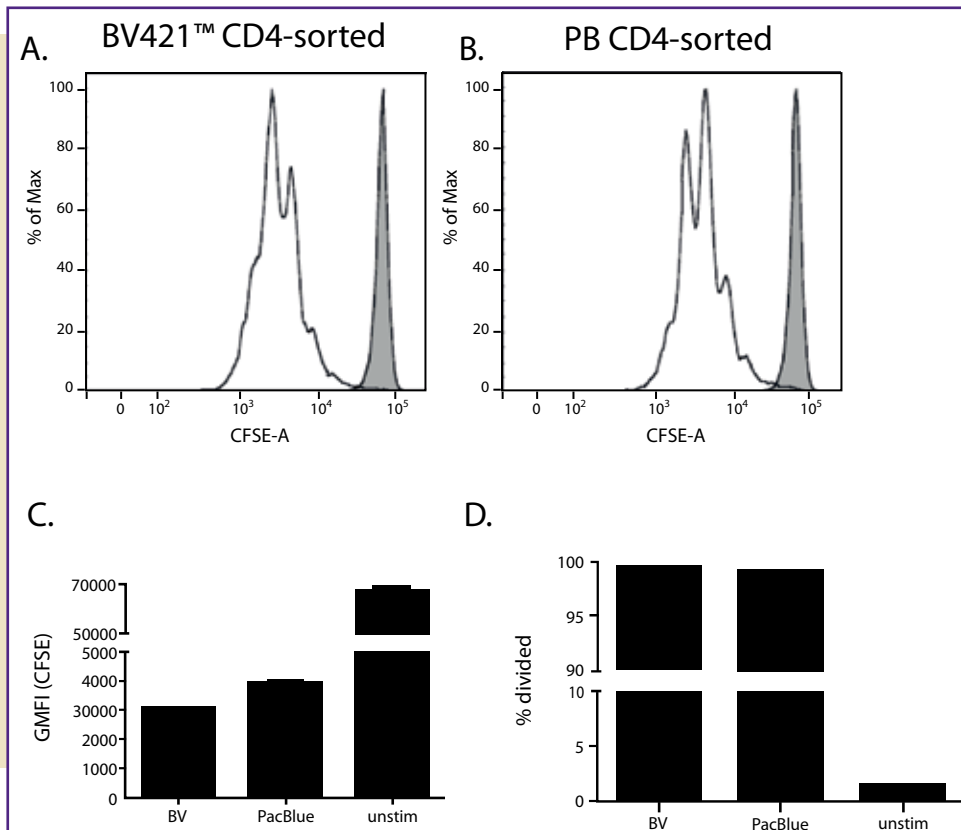
Cell viability is critical after cell sorting. Here, pooled Balb/c spleen and lymph node cells were stained with CD4-BV421™ or CD4-Pacific Blue™ and sorted for CD4 positive cells. The cells were then labeled with CFSE and stimulated in 96-well flat-bottom plates coated with anti-CD3/anti-CD28 (1 µg/ml each) at 1 x 10⁵ cells/well. On day four, cells were analyzed for CFSE dilution. Histograms A and B demonstrate comparable division and expansion of cells sorted with BV421™ compared to

those sorted with Pacific Blue™, as indicated by the loss of CFSE signal. Unstimulated cells are displayed as the gray histogram. Figure C shows the median fluorescence values of each group. Error bars represent the standard deviation of triplicate wells. Figure D demonstrates that the % of cells that have divided is comparable between BV421™ and Pacific Blue™-stained cells. Overall, the data shows excellent viability and responsiveness of cells after staining and sorting with a BV421™ antibody.

Data Courtesy of Rick Willis and John Altman, NIH Tetramer Core Facility at Emory/Yerkes.



Data provided by Aras Toker and Jochen Hühn, Helmholtz Centre for Infection Research.



Brilliant Violet 421™

Resolution of human CD56 populations

Cat. No.	Description	Clone
318327/8	Brilliant Violet 421™ anti-human CD56 (NCAM)	HCD56

Brilliant Resolution

Resolution is critical for identifying both rare cell populations and dimly expressed markers. For human NK cells, the labeling of CD56 is commonly used for flow cytometry to identify CD56-negative, CD56-positive and CD56-bright populations. Here, researchers demonstrate the resolving power of Brilliant Violet 421™, with

brightness on par with PE/Cy7-conjugated antibody, resulting in similar population percentages. The BV421™ antibody is able to clearly resolve the NK cell populations, providing far superior signal-to-noise compared to Pacific Blue™ antibody.

Multicolor Microscopy with human CD56

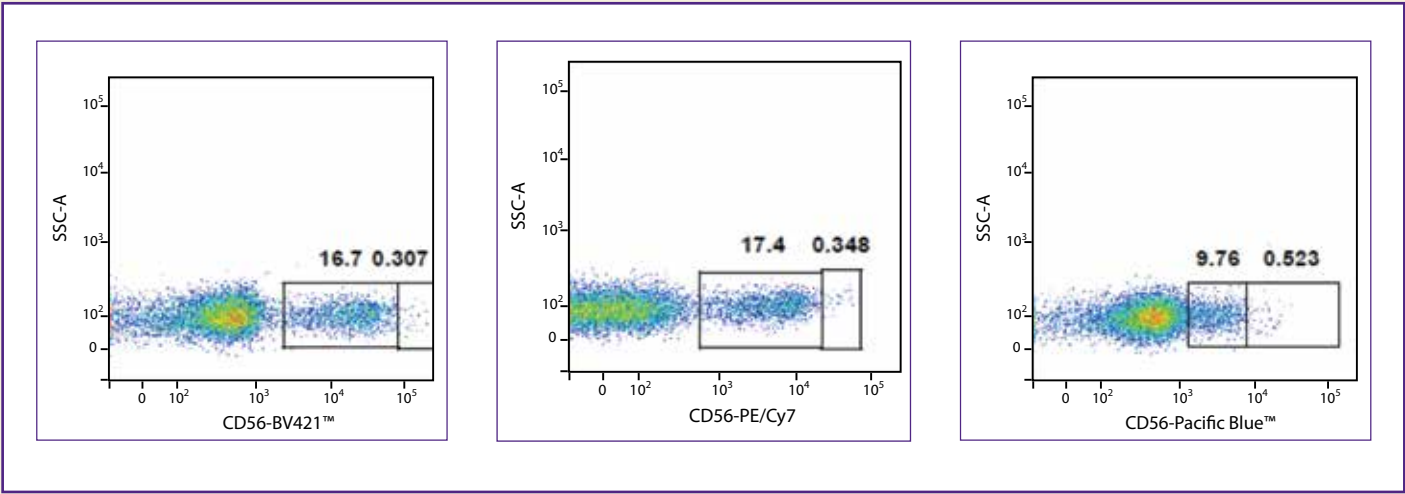
Cat. No.	Description	Clone
318327/8	Brilliant Violet 421™ anti-human CD56 (NCAM)	HCD56

Brilliant Imaging

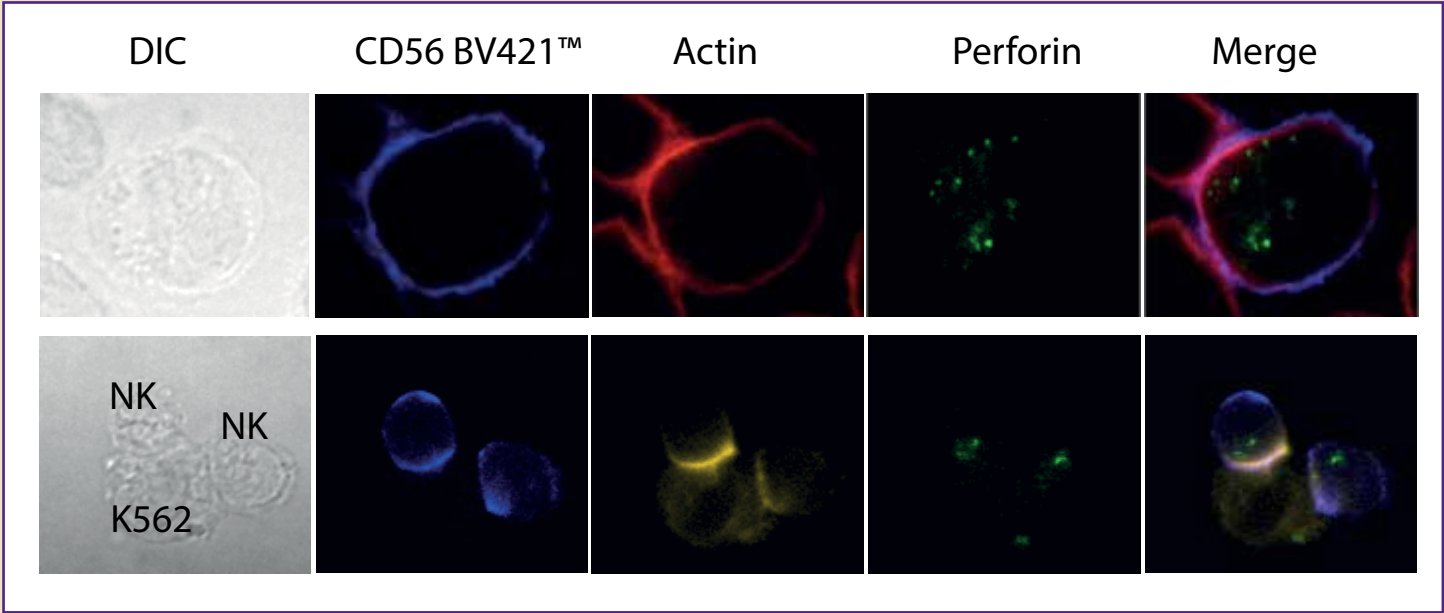
The high intensity brightness and exceptional photostability of Brilliant Violet 421™ enable for the first time, visualization of antigens using directly-labeled antibody conjugates in the “blue-emitting” channel for microscopy. Here, researchers stained NK92 cells (human NK cell line, top row) or NK92 cells mixed with K562 cells (bottom row) with anti-CD56 BV421™, anti-perforin

FITC, and phalloidin Alexa Fluor® 568, and acquired images on an Olympus IX81 spinning disk confocal microscope on 100X objective, NA 1.45. Exposures: 488 = 1000 ms, 568 = 100 ms, BV421™ (450 nm) = 200 ms. CD56-expressing NK cells were easily distinguished from the CD56-non-expressing K562 cells.

Data courtesy of Axel Schulz and Andreas Thiel at BCRT/Charite Berlin.



Data courtesy of Emily Mace and Jordan Orange, Children's Hospital of Philadelphia.



15 Color assay using Brilliant Violet™ fluorophores

Cat. No.	Description	Clone
317331/2	Brilliant Violet 510™ anti-human CD3	OKT3
301043	Brilliant Violet 711™ anti-human CD8a	RPA-T8
301833	Brilliant Violet 605™ anti-human CD14	M5E2
302235	Brilliant Violet 570™ anti-human CD19	HIB19
302827/8	Brilliant Violet 650™ anti-human CD27	O323
306017/8	Brilliant Violet 421™ anti-human CD123	6H6

Brilliant Construction

The Brilliant Violet™ fluorophores do spill over into neighboring channels off the violet laser. However, since spillover values are only one variable that can affect sensitivity in a multicolor panel, intelligently balancing a panel is the most important strategy for the maintenance of resolution in any channel. When two fluorophores exhibit significant spillover and are not of equal brightness, like BV570™ and BV605™, the best strategy to maintain resolution is to select markers that are not co-expressed on the same cell type, in

this instance, CD19 and CD14. Also involved in this balance is the assignment of a very bright fluorophore like BV605™ to a lowly-expressed antigen like CD14, and a dim fluorophore like BV570™ to an abundant antigen like CD19. The brightness of BV421™ and BV605™ and the significant expansion of commercial conjugate choices that are well balanced to antigen expression level make it easier for >12 color panels to become standard in basic and clinical research applications.

Cytokine profiling of T cell subsets

Cat. No.	Description	Clone
317331/2	Brilliant Violet 510™ anti-human CD3	OKT3
301043	Brilliant Violet 711™ anti-human CD8a	RPA-T8
302637	Brilliant Violet 785™ anti-human CD25	BC96
500331	Brilliant Violet 605™ anti-human IL-2	MQ1-17H12
512321/2	Brilliant Violet 421™ anti-human IL-17A	BL168
502537	Brilliant Violet 650™ anti-human IFN-γ	4S.B3

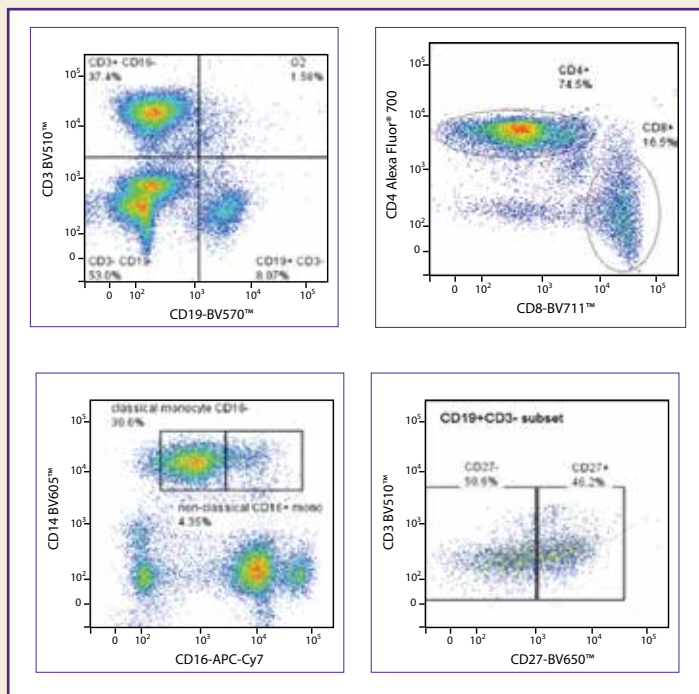
Brilliant Cytokine Staining

While quantum dots provide expanded options for colors on the violet laser, they do not perform well for intracellular staining and lack direct conjugate options for intracellular flow cytometry. In contrast to quantum dot nanocrystals, Brilliant Violet™-conjugated antibodies are applicable in a 13-color intracellular cytokine and FOXP3 panel for the detection of intracellular antigens (IL-17A, IFN-γ, IL-2), as well as for surface antigens (CD3, CD8, CD25),

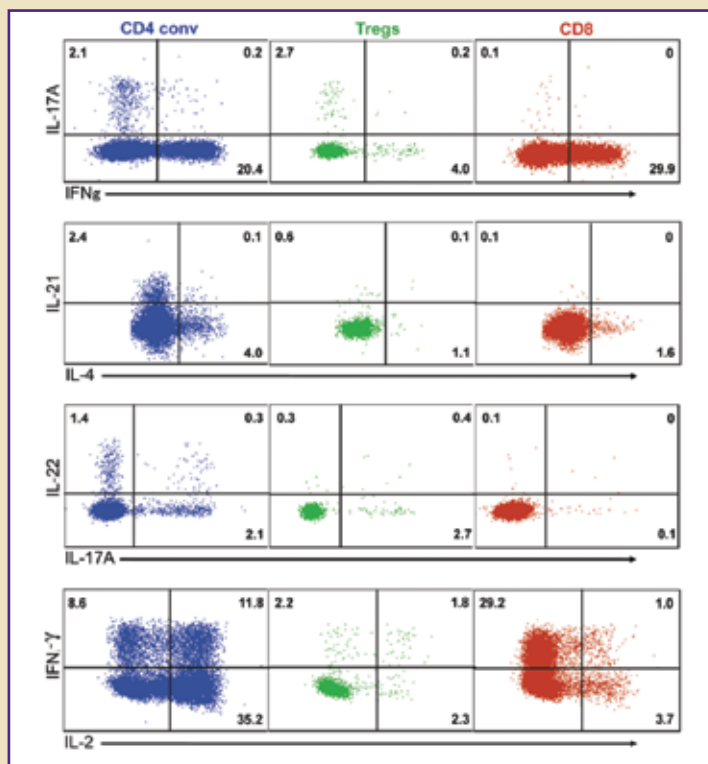
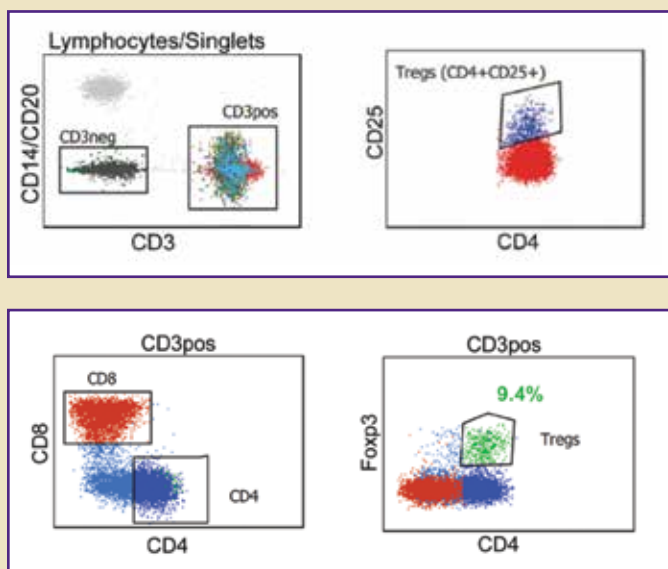
due to their stability following fixation and permeabilization. Thus, the novel family of Brilliant Violet™ fluorescent molecules BV421™, BV510™, BV605™, BV650™, BV711™, and BV785™ provide superior new tools for the violet laser line, further enhancing the capabilities for multicolor flow cytometry.

Fluorophore/PMT	Specificity
BV510™	CD3
PerCP/Cy5.5	CD4
BV711™	CD8a
Alexa Fluor® 700	CD14
BV785™	CD25
FITC	FOXP3
BV605™	IL-2
PE/Cy7	IL-4
BV421™	IL-17A
APC	IL-21
PE	IL-22
BV650™	IFN-γ
APC/Cy7	Live/Dead NIR

Fluorophore/PMT	Specificity
BV510™	CD3
Alexa Fluor® 700	CD4
BV711™	CD8a
PE/Cy5	CD11c
BV605™	CD14
APC/Cy7	CD16
BV570™	CD19
PerCP/Cy5.5	CD20
BV650™	CD27
FITC	CD38
PE/Cy7	CD45RA
PE	CD56
BV421™	CD123
APC	CD197
PE/CF594	HLA-DR



Data courtesy of by Eva Tolosa, University Medical Centre Hamburg-Eppendorf.



Intracellular Staining with anti-human IFN-γ

Cat. No.	Description	Clone
502533	Brilliant Violet 570™ anti-human IFN-γ	4S.B3

Brilliant Intracellular Staining

Intracellular staining of cytokines can be an excellent tool for identifying single cell polarization towards T helper phenotypes. With the recent discovery of many different T helper types, including Th1, Th2, Th17, Tfh, Th9, Th22, and IL-10 producing regulatory T cells, it is critical to delineate T cells using multicolor intracellular flow cytometry. Furthermore, functional characterization of other cell types, such as CD8⁺ cells, requires the detection of multiple intracellular cytokines. Here, we demonstrate that Brilliant Violet 570™ is fully compatible with other anti-cytokine antibodies, including IL-17A

Brilliant Violet 421™, in a 10-color panel using BioLegend's standard Fixation and Permeabilization buffers. The BV570™ antibody is able to clearly resolve the IFN-γ-expressing cells (Th1) from the Th2 and Th17 cells in a mixed human peripheral blood lymphocyte population, after stimulation with PMA/ionomycin for six hours. Positive gates were determined from FMO and unstimulated controls. The full panel and instrument configuration are shown to the right: (Note: Filter changes and instrument re-configuration may be necessary.)

9 - Color Immunophenotyping infiltrating Lymphocytes in Breast Cancer Tissue

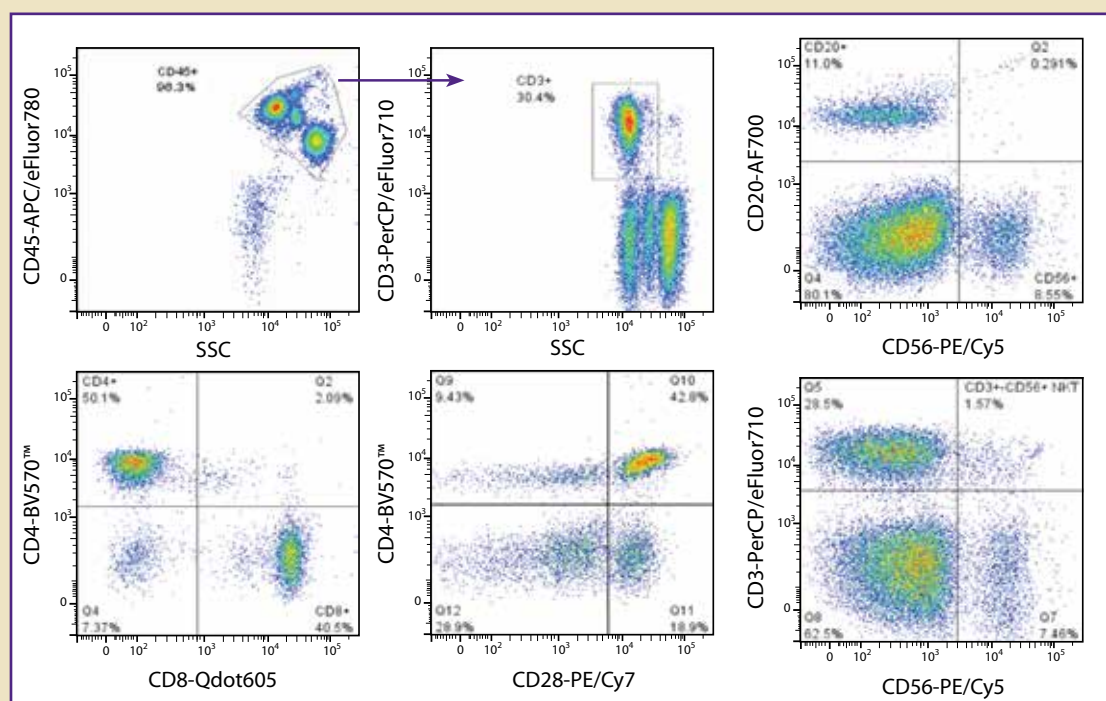
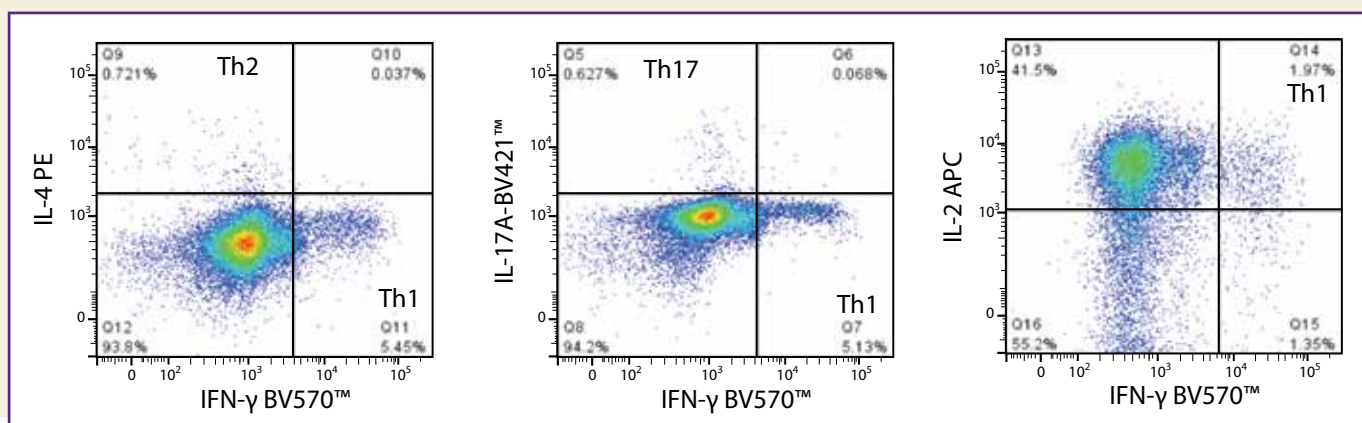
Cat. No.	Description	Clone
300533	Brilliant Violet 570™ anti-human CD4	RPA-T4

Brilliant Multicolor

A common application in cancer immunology involves profiling primary tumor specimens for tumor infiltrating leukocytes and their proliferation/activation states. Often, these sorts of profiling applications require many concurrent panels to cover phenotypic markers for granulocytes, T cells, B cells, NKT cells, myeloid and dendritic cells. Here, a breast cancer tissue specimen was assayed for T, B and NK cells in a single 9-color panel. Cells were gated on SSC vs. CD45 and then CD3⁺ populations. Brilliant

Violet 570™ is an additional marker excited by the violet laser that is brighter than its spectral equivalent, Pacific Orange™, and can be used in the same panel as BD Horizon™ V500 or Live/Dead Fixable Aqua and QDot® 605 in a multicolor panel. In this application, CD4-BV570™ is used in place of CD4-QDot® 655. The data at right demonstrates the utility of CD4-BV570™ as a drop-in replacement, in a 9-color panel. (Note: Filter changes and instrument re-configuration may be necessary.)

Fluorophore/PMT	Specificity	Laser	Voltage	Bandpass	Dichroic
APC	IL-2	633	459	670/30	
Alexa Fluor® 700	CD3	633	385	730/45	690LP
BV421™	IL-17A	405	368	450/50	
Aqua	Live/Dead	405	429	525/50	505LP
BV570™	IFN-γ	405	486	585/42	556LP
Qdot®605	CD8	405	409	605/40	595LP
Alexa Fluor® 488	CD45RO	488	489	530/30	505LP
PE	IL-4	561	419	582/15	
PE/Cy5	CD4	561	471	660/20	635LP
PE/Cy7	CD45RA	561	524	780/60	750LP



Fluorophore	Specificity
APC/eFluor®780	CD45
PerCP/eFluor®710	CD3e
eFluor®450	HLA-DR
BV570™	CD4
Qdot®605	CD8a
PE/Cy7	CD28
FITC	NKG2D
PE/Cy5	CD56
Alexa Fluor® 700	CD20
Aqua	Live/Dead

Brilliant Violet 570™

6-Color Treg Identification

Cat. No.	Description	Clone
351307	Brilliant Violet 570™ anti-human CD127	A019D5
302629/30	Brilliant Violet 421™ anti-human CD25	BC96

Brilliant Clarity

Flow cytometry is an effective tool for the identification of Treg (Regulatory T) cells. While FOXP3 is the definitive marker for Tregs, it is an intracellular target that requires fixation and permeabilization of cells for antibody labeling, which precludes researchers from isolating and performing further functional studies on those cells. CD127 and CD25 have been identified as useful cell surface markers for the classification of Tregs without the use of a FOXP3 antibody, characterized as CD127^{dim}/CD25⁺/CD4⁺ cells. Further characterization of Tregs requires

multicolor flow cytometry, potentially with many different labeled antibodies, increasing the need for more fluorophore options. Here, CD127-BV570™ was successfully used for PBMC staining with a 6-color panel, including CD25-BV421™, to identify Tregs based on cell surface staining. Other functional Treg markers, CD39 and CD73, which are cell surface ectonucleotidase enzymes that contribute to the suppressive capacity of Tregs, were also characterized in this panel. (Note: Filter changes and instrument re-configuration may be necessary.)

Brilliant Violet 605™

Comparison of Cytokine Expression on CD154⁺/CD8⁺ and CD154⁺/CD8⁻ cells

Cat. No.	Description	Clone
301039	Brilliant Violet 605™ anti-human CD8a	RPA-T8
310823	Brilliant Violet 421™ anti-human CD154	24-31
359605	Brilliant Violet 570™ anti-human CD57	HNK-1
302827/8	Brilliant Violet 650™ anti-human CD27	O323

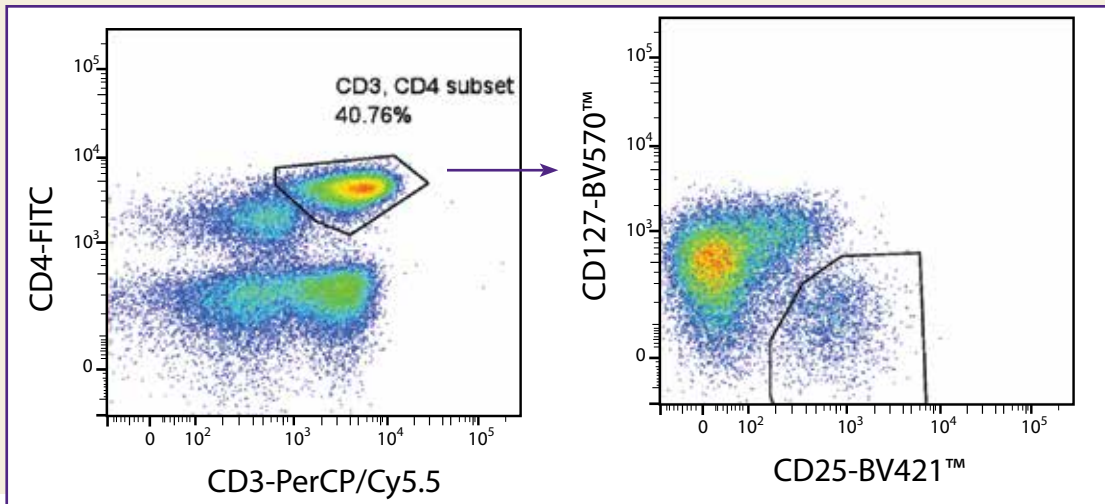
Brilliant Stability for Intracellular Staining

CD154 (CD40L) is upregulated in activated cells, where it interacts with CD40 to induce B-cell activation, cytokine production and thus, fate determination. For this experiment, T cell activation is determined by expression of IL-2 and IFN-γ. In this analysis, CD3 PerCP/Cy5.5 vs. CD8 BV605™ defines CD8⁺ vs. CD4⁺ (CD8⁻) T-cells. From those subsets, CD154 BV421™ vs. IL-2 Alexa Fluor® 700 or CD154 BV421™ vs. IFN-γ PE/Cy7 expression is compared. CD4⁺ cells express more CD154

and IL-2 than CD8⁺ cells, and IFN-γ expression is similar in both populations. IL-2 is strongly co-expressed with CD154, especially in CD4⁺ cells. The majority of IFN-γ in CD8⁺ cells is singly expressed, while in CD4⁺ cells, the majority of IFN-γ is co-expressed with CD154, due in part to the reduced CD154 expression in CD8⁺ cells. However, when comparing the ratio of CD154⁺ cells to CD154/IFN-γ co-expressing cells, CD154⁺, CD4⁺ and CD8⁺ T cells are roughly similar in their ability to produce IFN-γ.

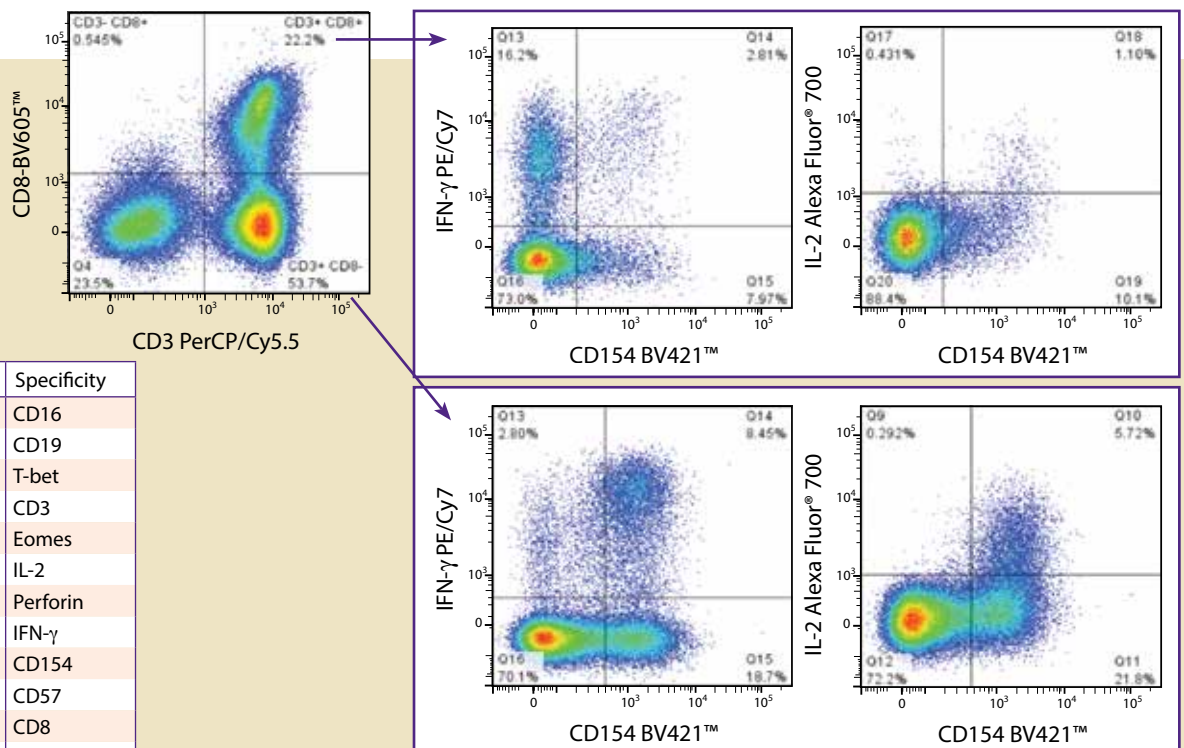
Data provided by Eva Tolosa, University Medical Center Hamburg-Eppendorf.

Fluorophore	Specificity
BV421™	CD25
BV570™	CD127
FITC	CD4
PerCP/Cy5.5	CD3
PE/Cy7	CD39
APC	CD73



Data provided by Morgan Reuter and Michael Betts, University of Pennsylvania

Fluorophore	Specificity
PE/Cy5	CD16
PE/Cy5	CD19
FITC	T-bet
PerCP/Cy5.5	CD3
AF647	Eomes
AF700	IL-2
PE	Perforin
PE/Cy7	IFN-γ
BV421™	CD154
BV570™	CD57
BV605™	CD8
BV650™	CD27
Live/Dead	Blue



Brilliant Violet 650™

Treg Panel using Brilliant Violet™ fluorophores

Cat. No.	Description	Clone
317435	Brilliant Violet 650™ anti-human CD4	OKT4
302629/30	Brilliant Violet 421™ anti-human CD25	BC96
301037	Brilliant Violet 570™ anti-human CD8a	RPA-T8

Brilliant Family

Definitive characterization of Treg cells typically requires a combination of cell surface and intracellular staining. Directly labeled antibodies against CD3, CD4, and CD25 are commonly used for cell surface markers on Tregs, while FOXP3 is a specific intracellular marker. Here, we demonstrate the utility of Brilliant Violet™ fluorophores, BV421™, BV570™, and BV650™, in a Treg staining panel. In the analysis to the right, live

cells are positively gated and then analyzed for CD8 vs. CD4 expression. CD4⁺ cells are then analyzed for FOXP3 vs. CD25. Fluorescence Minus One (FMO) data reveals the specificity of the FOXP3⁺CD25⁺ cells. Being able to utilize such an expanded set of fluorophores greatly expands the options for more complex analysis with 9 or more colors, allowing for more in-depth studies on the characteristics and functions of Tregs.

Brilliant Violet 605™/ Brilliant Violet 650™

T cell Activation Panel in Rhesus Macaque

Cat. No.	Description	Clone
301039	Brilliant Violet 605™ anti-human CD8a	RPA-T8
302041	Brilliant Violet 650™ anti-human CD16	3G8
301831	Brilliant Violet 570™ anti-human CD14	M5E2
302331	Brilliant Violet 570™ anti-human CD20	2H7
305623/4	Brilliant Violet 421™ anti-human CD95	DX2

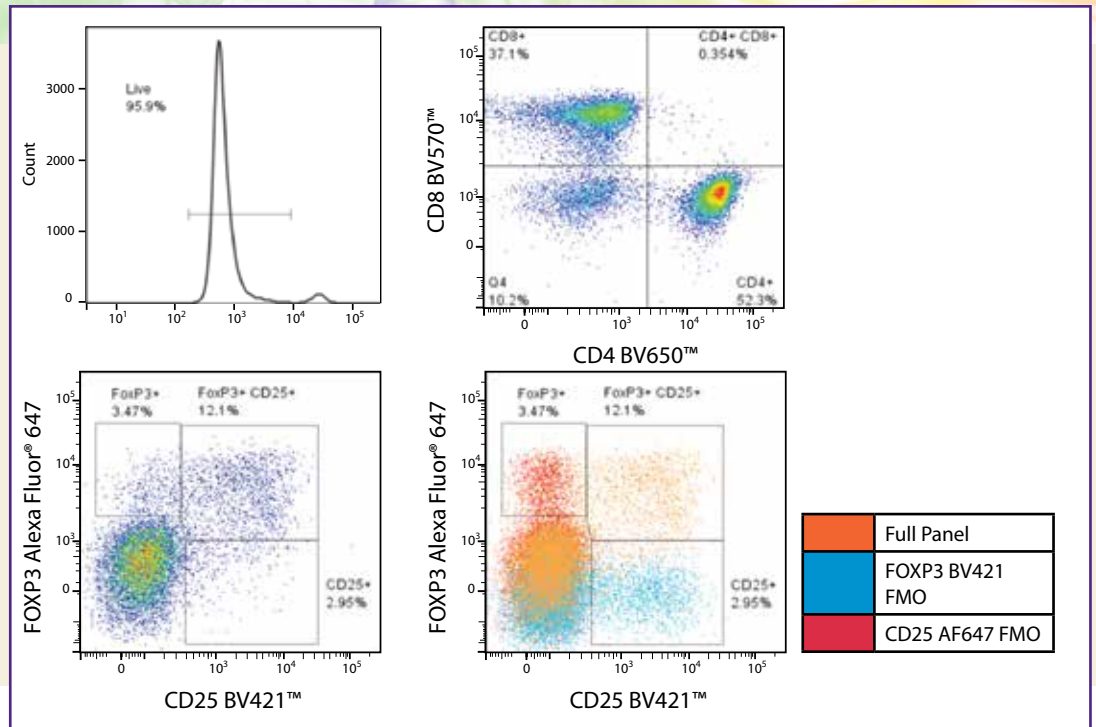
Brilliant Reactivity

Rhesus macaque is a common model for immune studies in response to pathogens. In this panel, T-cell activation was assessed in a variety of subsets. A 12 color flow panel was run on an LSRII (405, 488, and 633 nm lasers). On gated lymphocytes, dead cells were excluded, as well as CD16 BV650™ and CD14 and CD20 BV570™. From the enriched population, T cells were phenotyped into naïve, effector and memory subsets on the

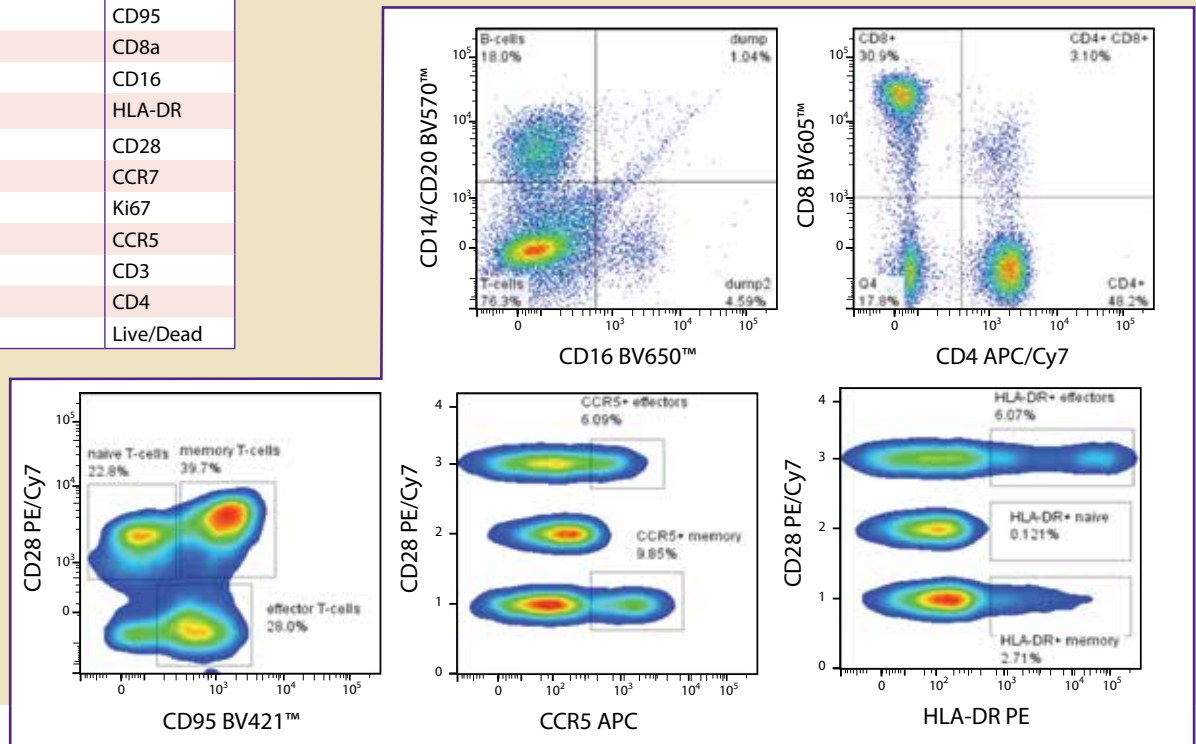
total T cell population, as well as broken into CD4⁺ and CD8⁺ selective populations by gating on CD28 and CD95. Naïve T cells are CD28⁺/CD95⁻, memory are CD28⁺/CD95⁺, and effector T cells are CD28⁻/CD95⁺. Each T cell subset was then assessed for the degree of expression of activation markers such as CCR5, HLA-DR, CCR7 and proliferation status with Ki-67.

Data provided by Albert Bendelac, Rachel Reboulet and David Leclerc,
University of Chicago, and Kelly Lundsten, BioLegend.

Fluorophore	Specificity
PE/Cy7	CD3
BV570™	CD8
BV421™	CD25
BV650™	CD4
Alexa Fluor® 647	FOXP3
Green	Live/Dead



Fluorophore	Specificity
BV570™	CD14/CD20
BV421™	CD95
BV605™	CD8a
BV650™	CD16
PE	HLA-DR
PE/Cy7	CD28
FITC	CCR7
PerCP/Cy5.5	Ki67
APC	CCR5
Alexa Fluor® 700	CD3
APC/Cy7	CD4
Aqua	Live/Dead



Brilliant Violet™ Products Available

Brilliant Violet 421™			
Specificity	Clone	Cat. No.	Size
Human			
CD1a	HI149	300127	25 tests
CD1c	L161	331525 331526	25 tests 100 tests
CD2	TS1/8	309217 309218	25 tests 100 tests
CD3	UCHT1	300433 300434	25 tests 100 tests
CD4	RPA-T4	300531 300532	25 tests 100 tests
CD4	OKT4	317433 317434	25 tests 100 tests
CD5	UCHT2	300625 300626	25 tests 100 tests
CD8a	RPA-T8	301035 301036	25 tests 100 tests
CD10	HI10a	312217 312218	25 tests 100 tests
CD11b	ICRF44	301323 301324	25 tests 100 tests
CD11b	M1/70	101235 101236	125 µl 500 µl
CD11c	3.9	301627 301628	25 tests 100 tests
CD14	M5E2	301829 301830	25 tests 100 tests
CD16	3G8	302037 302038	25 tests 100 tests
CD19	HIB19	302233 302234	25 tests 100 tests
CD20	2H7	302329 302330	25 tests 100 tests
CD24	ML5	311121 311122	25 tests 100 tests
CD25	BC96	302629 302630	25 tests 100 tests
CD27	O323	302823 302824	25 tests 100 tests
CD28	CD28.2	302929 302930	25 tests 100 tests
CD31	WM59	303123	25 tests
CD33	WM53	303415 303416	25 tests 100 tests
CD34	561	343609 343610	25 tests 100 tests
CD38	HIT2	303525 303526	25 tests 100 tests
CD39	A1	328213	25 tests
CD44	BJ18	338809 338810	25 tests 100 tests
CD45	HI30	304031 304032	25 tests 100 tests
CD45RA	HI100	304129 304130	25 tests 100 tests
CD45R/B220	RA3-6B2	103239 103240	125 µl 500 µl
CD45RO	UCHL1	304223	25 tests

Brilliant Violet 421™			
Specificity	Clone	Cat. No.	Size
Human			
CD56 (NCAM)	HCD56	318327 318328	25 tests 100 tests
CD62L	DREG-56	304827	25 tests
CD64	10.1	305019 305020	25 tests 100 tests
CD69	FN50	310929 310930	25 tests 100 tests
CD73	AD2	344007 344008	25 tests 100 tests
CD80	2D10	305221	25 tests
CD83	HB15e	305323 305324	25 tests 100 tests
CD86	IT2.2	305425 305426	25 tests 100 tests
CD90 (Thy1)	5E10	328121 328122	25 tests 100 tests
CD95 (Fas)	DX2	305623 305624	25 tests 100 tests
CD105	43A3	323219	25 tests
CD107a (LAMP-1)	H4A3	328625 328626	25 tests 100 tests
CD117 (c-kit)	104D2	313215 313216	25 tests 100 tests
CD122 (IL-2Rβ)	TU27	339009 339010	25 tests 100 tests
CD123	6H6	306017 306018	25 tests 100 tests
CD127 (IL-7Ra)	A019D5	351309 351310	25 tests 100 tests
CD134	Ber-ACT35 (ACT35)	350013	25 tests
CD137 (4-1BB)	4B4-1	309819	25 tests
CD154	24-31	310823	25 tests
CD158e1 (KIR3DL1, NKB1)	DX9	312713 312714	25 tests 100 tests
CD161	HP-3G10	339913 339914	25 tests 100 tests
CD183	G025H7	353715 353716	25 tests 100 tests
CD184 (CXCR4)	12G5	306517 306518	25 tests 100 tests
CD185 (CXCR5)	J252D4	356919 356920	25 tests 100 tests
CD192 (CCR2)	K036C2	357209	25 tests
CD193 (CCR3)	5E8	310713 310714	25 tests 100 tests
CD194 (CCR4)	L291H4	359413 359414	25 tests 100 tests
CD196	G034E3	353407 353408	25 tests 100 tests
CD197 (CCR7)	G043H7	353207 353208	25 tests 100 tests
CD200 (OX2)	OX-104	329209	25 tests
CD203c (E-NPP3)	NP4D6	324611	25 tests
CD274 (B7-H1, PD-L1)	29E.2A3	329713	25 tests

For the latest product updates, visit: biolegend.com

Brilliant Violet™ Products Available

Brilliant Violet 421™			
Specificity	Clone	Cat. No.	Size
Human			
CD279 (PD-1)	EH12.2H7	329919 329920	25 tests 100 tests
CD284 (TLR4)	HTA125	312811	25 tests
CD294 (CRTH2)	BM16	350111 350112	25 tests 100 tests
CD303 (BDCA-2)	201A	354211	25 tests
CD304 (Neuropilin-1)	12C2	354513	25 tests
CD326 (EpCAM)	9C4	324219	25 tests
CD335 (NKp46)	9E2	331913	25 tests
CD360 (IL-21R)	2G1-K12	347809	25 tests
EGFR	AY13	352911	25 tests
GARP (LRRC32)	7B11	352509	25 tests
HLA-DR	L243	307635 307636	25 tests 100 tests
IFN-γ	4S.B3	502531 502532	25 tests 100 tests
Ig light chain κ	MHK-49	316517 316518	25 tests 100 tests
IgD	IA6-2	348225 348226	25 tests 100 tests
IgG Fc	HP6017	409317 409318	25 tests 100 tests
IgM	MHM-88	314515 314516	25 tests 100 tests
IL-2	MQ1-17H12	500327 500328	25 tests 100 tests
IL-4	MP4-25D2	500825 500826	25 tests 100 tests
IL-10	JES3-9D7	501421	25 tests
IL-17A	BL168	512321 512322	25 tests 100 tests
Ki-67	Ki-67	350505	25 tests
LAP (TGF-β1)	TW4-2F8	349613	25 tests
Perforin	B-D48	353307	25 tests
T-bet	4B10	644815 644816	125 µl 500 µl
TCR α/β	IP26	306721	25 tests
TCR γ/δ	B1	331217 331218	25 tests 100 tests
Tim-3	F38-2E2	345007 345008	25 tests 100 tests
TNF-α	MAB11	502931 502932	25 tests 100 tests

Brilliant Violet 421™			
Specificity	Clone	Cat. No.	Size
Mouse			
CD3	17A2	100227	125 µl
CD3ε	145-2C11	100335 100336	125 µl 500 µl
CD4	GK1.5	100437 100438	125 µl 500 µl

For the latest product updates, visit: biolegend.com

Brilliant Violet 421™			
Specificity	Clone	Cat. No.	Size
Mouse			
CD4	RM4-5	100543 100544	125 µl 500 µl
CD5	53-7.3	100617 100618	125 µl 500 µl
CD8a	53-6.7	100737 100738	125 µl 500 µl
CD11b	M1/70	101235 101236	125 µl 500 µl
CD11c	N418	117329 117330	125 µl 500 µl
CD16/32	93	101331	125 µl
CD19	6D5	115537 115538	125 µl 500 µl
CD21/CD35 (CR2/CR1)	7E9	123421	125 µl
CD24	M1/69	101825	125 µl
CD25	PC61	102033 102034	125 µl 500 µl
CD31	390	102423	125 µl
CD34	MEC14.7	119321	125 µl
CD41	MWReg30	133911	125 µl
CD44	IM7	103039	125 µl
CD45	30-F11	103133	125 µl
CD45.1	A20	110731	125 µl
CD45.2	104	109831	125 µl
CD45R/B220	RA3-6B2	103239 103240	125 µl 500 µl
CD48	HM48-1	103427	125 µl
CD62L	MEL-14	104435	125 µl
CD69	H1.2F3	104527 104528	125 µl 500 µl
CD71	RI7217	113813	125 µl
CD80	16-10A1	104725 104726	125 µl 500 µl
CD86	GL-1	105031	125 µl
CD103	2E7	121421	125 µl
CD107a (LAMP-1)	1D4B	121617	125 µl
CD117 (c-kit)	2B8	105827 105828	125 µl 500 µl
CD117 (c-kit)	ACK2	135123 135124	125 µl 500 µl
CD127	A7R34	135023 135024	125 µl 500 µl
CD134 (OX-40)	OX-86	119411	125 µl
CD135	A2F10	135313 135314	125 µl 500 µl
CD138 (Syndecan-1)	281-2	142507 142508	125 µl 500 µl
CD150 (SLAMF)	TC15-12F12.2	115925 115926	125 µl 500 µl
CD152	UC10-4B9	106311	125 µl
CD183 (CXCR3)	CXCR3-173	126521 126522	125 µl 500 µl
CD185 (CXCR5)	L138D7	145511 145512	125 µl 500 µl
CD194 (CCR4)	2G12	131217	125 µl

Brilliant Violet™ Products Available

Brilliant Violet 421™			
Specificity	Clone	Cat. No.	Size
Mouse (continued)			
CD196 (CCR6)	29-2L17	129817 129818	125 µl 500 µl
CD197 (CCR7)	4B12	120119 120120	125 µl 500 µl
CD206 (MMR)	C068C2	141717	125 µl
CD274 (B7-H1, PD-L1)	10F9G2	124315	125 µl
CD279 (PD-1)	29F.1A12	135217 135218	125 µl 500 µl
CD304 (Neuropilin-1)	3E12	145209	125 µl
CD335 (NKP46)	29A1.4	137611	125 µl
F4/80	BM8	123131 123132	125 µl 500 µl
I-A/I-E	M5/114.15.2	107631	125 µl
IFN-γ	XMG1.2	505829	125 µl
IgM	RMM-1	406517 406518	125 µl 500 µl
IL-2	JES6-5H4	503825	125 µl
IL-4	11B11	504119 504120	125 µl 500 µl
IL-10	JES5-16E3	505021	125 µl
IL-17A	TC11-18H10.1	506925	125 µl
KLRG1	2F1/KLRG1	138413	125 µl
LAP (TGF-β1)	TW7-16B4	141407	125 µl
Ly-6A/E (Sca-1)	D7	108127	125 µl
Ly-6C	HK1.4	128031 128032	125 µl 500 µl
Ly-6G	1A8	127627	125 µl
Ly-6G/Ly-6C (Gr-1)	RB6-8C5	108433 108434	125 µl 500 µl
NK-1.1	PK136	108731 108732	125 µl 500 µl
Notch 1	HMN1-12	130615	125 µl
T-bet	4B10	644815 644816	125 µl 500 µl
TCR-β Chain	H57-597	109229	125 µl
TCR γ/δ	GL3	118119	125 µl
TER-119/ErythroidCells	TER-119	116233	125 µl
TNF-α	MP6-XT22	506327	125 µl

Brilliant Violet 421™			
Specificity	Clone	Cat. No.	Size
Rat			
CD90.1	OX-7	202529	125 µl

Brilliant Violet 421™			
	Clone	Cat. No.	Size
Secondary Reagents			
Goat Anti-Mouse IgG	Poly4053	405317	125 µl
Goat Anti-Rat IgG	Poly4054	405414	125 µl
Donkey Anti-Rabbit IgG	Poly4064	406410	125 µl
Streptavidin-BV421™		405225 405226	100 µg 100 µl

For the latest product updates, visit: biolegend.com

Brilliant Violet 421™			
	Clone	Cat. No.	Size
Isotype Controls			
Armenian Hamster IgG	HTK888	400935 400936	125 µl 500 µl
Mouse IgG1, κ	MOPC-21	400157 400158	25 tests 100 tests
Mouse IgG2a, κ	MOPC-173	400259 400260	25 tests 100 tests
Mouse IgG2b, κ	MPC-11	400341 400342	25 tests 100 tests
Rat IgG1, κ	RTK2071	400429 400430	125 µl 500 µl
Rat IgG2a, κ	RTK2758	400535 400536	125 µl 500 µl
Rat IgG2b, κ	RTK4530	400639 400640	125 µl 500 µl

Brilliant Violet 510™			
Specificity	Clone	Cat. No.	Size
Human			
CD3	OKT3	317331 317332	25 tests 100 tests
CD4	OKT4	317443 317444	25 tests 100 tests
CD8a	RPA-T8	301047 301048	25 tests 100 tests
CD10	HI10a	312219	25 tests
CD11b	ICRF44	301333 301334	25 tests 100 tests
CD11c	3.9	301633	25 tests
CD14	M5E2	301841 301842	25 tests 100 tests
CD15 (SSEA-1)	W6D3	323027	25 tests
CD16	3G8	302047 302048	25 tests 100 tests
CD19	HIB19	302241 302242	25 tests 100 tests
CD20	2H7	302339 302340	25 tests 100 tests
CD25	M-A251	356119	25 tests
CD27	O323	302835 302836	25 tests 100 tests
CD28	CD28.2	302935 302936	25 tests 100 tests
CD34	5B1	343527	25 tests
CD38	HB-7	356611	25 tests
CD45	HI30	304035 304036	25 tests 100 tests
CD45RA	HI100	304141 304142	25 tests 100 tests
CD49d	9F10	304317	25 tests
CD56 (NCAM)	HCD56	318339 318340	25 tests 100 tests
CD69	FN50	310935	25 tests
CD86	IT2.2	305431 305432	25 tests 100 tests
CD90 (Thy1)	5E10	328125	25 tests

Brilliant Violet™ Products Available

Brilliant Violet 510™			
Specificity	Clone	Cat. No.	Size
Human			
CD117 (c-kit)	104D2	313219	25 tests
CD123	6H6	306021 306022	25 tests 100 tests
CD127 (IL-7Rα)	A019D5	351331 351332	25 tests 100 tests
CD183 (CXCR3)	G025H7	353725	25 tests
CD185 (CXCR5)	J252D4	356921 356922	25 tests 100 tests
CD194 (CCR4)	L291H4	359415 359416	25 tests 100 tests
CD196 (CCR6)	G034E3	353423	25 tests
CD197 (CCR7)	G043H7	353231	25 tests
CD279 (PD-1)	EH12.2H7	329931 329932	25 tests 100 tests
CD335 (Nkp46)	9E2	331923	25 tests
HLA-DR	L243	307645 307646	25 tests 100 tests
IFN-γ	4S.B3	502543	25 tests
IgD	IA6-2	348219 348220	25 tests 100 tests
IgM	MHM-88	314521	25 tests
Ki-67	Ki-67	350517	25 tests

Brilliant Violet 510™			
Specificity	Clone	Cat. No.	Size
Mouse			
CD3	17A2	100233	125 µl
CD4	RM4-5	100553	125 µl
CD8a	53-6.7	100751	125 µl
CD11b	M1/70	101245	125 µl
CD11c	N418	117337 117338	125 µl 500 µl
CD19	6D5	115545 115546	125 µl 500 µl
CD25	PC61	102041 102042	125 µl 500 µl
CD44	IM7	103043	125 µl
CD45	30-F11	103137	125 µl
CD45.2	104	109837	125 µl
CD45R/B220	RA3-6B2	103247	125 µl
CD62L	MEL-14	104441	125 µl
CD69	H1.2F3	104531 104532	125 µl 500 µl
CD86	GL-1	105039 105040	125 µl 500 µl
CD90.2	53-2.1	140319	125 µl
CD117 (c-kit)	ACK2	135119	125 µl
CD150 (SLAMF)	TC15-12F12.2	115929	125 µl
CD183 (CXCR3)	CXCR3-173	126527	125 µl
I-A/I-E	M5/114.15.2	107635	125 µl
IFN-γ	XMG1.2	505841	125 µl
Ly-6A/E (Sca-1)	D7	108129	125 µl

Brilliant Violet 510™			
Specificity	Clone	Cat. No.	Size
Mouse			
Ly-6C	HK1.4	128033	125 µl
Ly-6G/Ly-6C (Gr-1)	RB6-8C5	108437 108438	125 µl 500 µl
NK-1.1	PK136	108737 108738	125 µl 500 µl
TCR-β chain	H57-597	109233 109234	125 µl 500 µl
TER-119/Erythroid Cells	TER-119	116237	125 µl

Brilliant Violet 510™			
	Clone	Cat. No.	Size
Isotype Controls and Secondary Reagents			
Armenian Hamster IgG	HTK888	400941	125 µl
Mouse IgG1, κ	MOPC-21	400171 400172	25 tests 100 tests
Mouse IgG2b, κ	MPC-11	400345 400346	25 tests 100 tests
Mouse IgG2a, κ	MOPC-173	400267	25 tests
Rat IgG1, κ	RTK2071	400435	125 µl
Rat IgG2a, κ	RTK2758	400547 400548	125 µl 500 µl
Rat IgG2b, κ	RTK4530	400645	125 µl
Streptavidin-BV510™		405233	100 µl

Brilliant Violet 570™			
Specificity	Clone	Cat. No.	Size
Human			
CD3	UCHT1	300435 300436	25 tests 100 tests
CD4	OKT4	317445	25 tests
CD4	RPA-T4	300533	25 tests
CD8a	RPA-T8	301037	25 tests
CD11b	ICRF44	301325	25 tests
CD14	M5E2	301831	25 tests
CD16	3G8	302035	25 tests
CD19	HIB19	302235	25 tests
CD20	2H7	302331	25 tests
CD27	O323	302825	25 tests
CD33	WM53	303417	25 tests
CD45	HI30	304033	25 tests
CD45RA	HI100	304131	25 tests
CD45RO	UCHL1	304225	25 tests
CD56 (NCAM)	HCD56	318329	25 tests
CD57	HNK-1	359605	25 tests
CD127 (IL-7Rα)	A019D5	351307	25 tests
HLA-DR	L243	307637	25 tests
IFN-γ	4S.B3	502533	25 tests
IgM	MHM-88	314517	25 tests
IL-17A	BL168	512323	25 tests

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Brilliant Violet™ Products Available

Brilliant Violet 570™			
Specificity	Clone	Cat. No.	Size
Mouse			
CD3	17A2	100225	125 µl
CD4	RM4-5	100541	125 µl
CD8a	53-6.7	100739	125 µl
CD11b	M1/70	101233	125 µl
CD11c	N418	117331	125 µl
CD19	6D5	115535	125 µl
CD44	IM7	103037	125 µl
CD45	30-F11	103135	125 µl
CD45.1	A20	110733	125 µl
CD45.2	104	109833	125 µl
CD45R/B220	RA3-6B2	103237	125 µl
CD62L	MEL-14	104433	125 µl
CD90.2	30-H12	105329	125 µl
Ly-6C	HK1.4	128029	125 µl
Ly-6G	1A8	127629	125 µl
Ly-6G/Ly-6C (Gr-1)	RB6-8C5	108431	125 µl
NK-1.1	PK136	108733	125 µl
TCR-β chain	H57-597	109231	125 µl

Brilliant Violet 570™			
Specificity	Clone	Cat. No.	Size
Rat			
CD90.1	OX-7	202531	125 µl

Brilliant Violet 570™			
	Clone	Cat. No.	Size
Isotype Controls and Secondary Reagents			
Armenian Hamster IgG	HTK888	400937	125 µl
Mouse IgG1, κ	MOPC-21	400159	25 tests
Mouse IgG2a, κ	MOPC-173	400261	25 tests
Mouse IgG2b, κ	MPC-11	400343	25 tests
Rat IgG2a, κ	RTK2758	400537	125 µl
Rat IgG2b, κ	RTK4530	400641	125 µl
Streptavidin-BV570™		405227	100 µl

Brilliant Violet 605™			
Specificity	Clone	Cat. No.	Size
Human			
CD3	OKT3	317321 317322	25 tests 100 tests
CD4	OKT4	317437 317438	25 tests 100 tests
CD8a	RPA-T8	301039	25 tests
CD10	HI10a	312221 312222	25 tests 100 tests
CD11b	ICRF44	301331 301332	25 tests 100 tests

Brilliant Violet 605™			
Specificity	Clone	Cat. No.	Size
Human			
CD11c	3.9	301635	25 tests
CD14	M5E2	301833	25 tests
CD15 (SSEA-1)	W6D3	323031 323032	25 tests 100 tests
CD16	3G8	302039	25 tests
CD19	HIB19	302243 302244	25 tests 100 tests
CD20	2H7	302333	25 tests
CD24	ML5	311123 311124	25 tests 100 tests
CD25	BC96	302631 302632	25 tests 100 tests
CD27	O323	302829 302830	25 tests 100 tests
CD31	WM59	303121 303122	25 tests 100 tests
CD34	581	343529	25 tests
CD38	HIT2	303531	25 tests
CD45	HI30	304041 304042	25 tests 100 tests
CD45RA	HI100	304133	25 tests
CD45RO	UCHL1	304237 304238	25 tests 100 tests
CD56 (NCAM)	HCD56	318333 318334	25 tests 100 tests
CD62L	DREG-56	304833 304834	25 tests 100 tests
CD69	FN50	310937 310938	25 tests 100 tests
CD80	2D10	305225	25 tests
CD86	IT2.2	305429	25 tests
CD90 (Thy1)	5E10	328127 328128	25 tests 100 tests
CD95 (Fas)	DX2	305627	25 tests
CD117 (c-kit)	104D2	313217 313218	25 tests 100 tests
CD123	6H6	306025	25 tests
CD127 (IL-7Ra)	A019D5	351333 351334	25 tests 100 tests
CD154	24-31	310825	25 tests
CD161	HP-3G10	339915	25 tests
CD183 (CXCR3)	G025H7	353727	25 tests
CD184 (CXCR4)	12G5	306521	25 tests
CD196 (CCR6)	G034E3	353419 353420	25 tests 100 tests
CD197 (CCR7)	G043H7	353223 353224	25 tests 100 tests
CD203c (E-NPP3)	NP4D6	324619	25 tests
CD279 (PD-1)	EH12.2H7	329923	25 tests
CD335 (NKp46)	9E2	331925	25 tests
HLA-DR	L243	307639 307640	25 tests 100 tests

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Brilliant Violet™ Products Available

Brilliant Violet 605™			
Specificity	Clone	Cat. No.	Size
Human			
IFN-γ	4S.B3	502535	25 tests
IgM	MHM-88	314523 314524	25 tests 100 tests
IL-2	MQ1-17H12	500331	25 tests
IL-4	MP4-25D2	500827	25 tests
IL-17A	BL168	512325	25 tests
Ki-67	Ki-67	350521	25 tests
Tim-3	F38-2E2	345017	25 tests
TNF-α	MAB11	502935	25 tests

Brilliant Violet 605™			
Specificity	Clone	Cat. No.	Size
Mouse			
CD3	17A2	100237	125 µl
CD4	RM4-5	100547	125 µl
CD8a	53-6.7	100743	125 µl
CD11b	M1/70	101237	125 µl
CD11c	N418	117333	125 µl
CD19	6D5	115539	125 µl
CD25	PC61	102035	125 µl
CD45	30-F11	103139 103140	125 µl 500 µl
CD45.1	A20	110737	125 µl
CD45R/B220	RA3-6B2	103243	125 µl
CD62L	MEL-14	104437	125 µl
CD69	H1.2F3	104529	125 µl
CD73	TY/11.8	127215	125 µl
CD80	16-10A1	104729	125 µl
CD86	GL-1	105037	125 µl
CD90.2 (Thy-1.2)	53-2.1	140317	125 µl
CD117 (c-kit)	ACK2	135121 135122	125 µl 500 µl
CD127 (IL-7Ra)	A7R34	135025	125 µl
CD138 (Syndecan-1)	281-2	142515 142516	125 µl 500 µl
CD150 (SLAMF)	TC15-12F12.2	115927	125 µl
CD183 (CXCR3)	CXCR3-173	126523	125 µl
CD185 (CXCR5)	L138D7	145513	125 µl
CD206 (MMR)	C068C2	141721	125 µl
CD279 (PD-1)	29F.1A12	135219	125 µl
F4/80	BM8	123133	125 µl
IFN-γ	XMG1.2	505839	125 µl
IL-2	JES6-5H4	503829	125 µl
IL-4	11B11	504125	125 µl
IL-17A	TC11-18H10.1	506927	125 µl
Ly-6A/E (Sca-1)	D7	108133	125 µl
Ly-6C	HK1.4	128035 128036	125 µl 500 µl
Ly-6G/Ly-6C (Gr-1)	RB6-8C5	108439	125 µl
NK-1.1	PK136	108739 108740	125 µl 500 µl
TER-119/Erythroid Cells	TER-119	116239	50 µg
TNF-α	MP6-XT22	506329	125 µl

Brilliant Violet 605™			
Specificity	Clone	Cat. No.	Size
Isotype Controls and Secondary Reagents			
Armenian Hamster IgG	HTK888	400943	125 µl
Mouse IgG1, κ	MOPC-21	400161 400162	25 tests 100 tests
Mouse IgG2a, κ	MOPC-173	400269	25 tests
Mouse IgG2b, κ	MPC-11	400349 400350	25 tests 100 tests
Rat IgG1, κ	RTK2071	400433	125 µl
Rat IgG2a, κ	RTK2758	400539	125 µl
Rat IgG2b, κ	RTK4530	400649 400650	125 µl 500 µl
Streptavidin-BV605™		405229	100 µl

Brilliant Violet 650™			
Specificity	Clone	Cat. No.	Size
Human			
CD3	OKT3	317323	25 tests
CD4	OKT4	317435	25 tests
CD8a	RPA-T8	301041	25 tests
CD11c	3.9	301637	25 tests
CD14	M5E2	301835	25 tests
CD16	3G8	302041	25 tests
CD19	HIB19	302237 302238	25 tests 100 tests
CD20	2H7	302335	25 tests
CD25	BC96	302633	25 tests
CD27	O323	302827 302828	25 tests 100 tests
CD45RA	HI100	304135	25 tests
CD45RO	UCHL1	304231	25 tests
CD56 (NCAM)	HCD56	318343	25 tests
CD62L	DREG-56	304831	25 tests
CD69	FN50	310933	25 tests
CD80	2D10	305227	25 tests
CD86	IT2.2	305427	25 tests
CD123	6H6	306019	25 tests
CD127 (IL-7Ra)	A019D5	351325 351326	25 tests 100 tests
CD196 (CCR6)	G034E3	353425	25 tests
CD197 (CCR7)	G043H7	353233	25 tests
CD335 (NKp46)	9E2	331927 331928	25 tests 100 tests
IFN-γ	4S.B3	502537	25 tests
IL-2	MQ1-17H12	500333	25 tests
TNF-α	MAB11	502937	25 tests

Brilliant Violet 650™			
Specificity	Clone	Cat. No.	Size
Mouse			
CD3	17A2	100229	125 µl
CD4	RM4-5	100545 100546	125 µl 500 µl
CD8a	53-6.7	100741	125 µl
CD11b	M1/70	101239	125 µl
CD11c	N418	117339	50 µg

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Brilliant Violet™ Products Available

Brilliant Violet 650™			
Specificity	Clone	Cat. No.	Size
Mouse (continued)			
CD19	6D5	115541	125 µl
CD25	PC61	102037	125 µl
CD45.1	A20	110735	125 µl
CD45.2	104	109835	125 µl
CD45R/B220	RA3-6B2	103241	125 µl
CD80	16-10A1	104731	125 µl
CD83	Michel-19	121515	125 µl
CD86	GL-1	105035	125 µl
CD117 (c-kit)	ACK2	135125	50 µg
CD138 (Syndecan-1)	281-2	142517	125 µl
CD150 (SLAMF)	TC15-12F12.2	115931	125 µl
CD206 (MMR)	C068C2	141723	50 µg
IFN-γ	XMG1.2	505831	125 µl
IL-17A	TC11-18H10.1	506929	125 µl
Ly-6G/Ly-6C (Gr-1)	RB6-8C5	108441	125 µl
NK-1.1	PK136	108735	125 µl
TER-119	TER-119	116235	125 µl

Brilliant Violet 650™			
	Clone	Cat. No.	Size
Isotype Controls and Secondary Reagents			
Armenian Hamster IgG	HTK888	400945	50 µg
Mouse IgG1, κ	MOPC-21	400163	25 tests
Mouse IgG2a, κ	MOPC-173	400265	25 tests
Mouse IgG2b, κ	MPC-11	400351	25 tests
Rat IgG1, κ	RTK2071	400437	125 µl
Rat IgG2a, κ	RTK2758	400541	125 µl
Rat IgG2b, κ	RTK4530	400651	50 µg
Streptavidin-BV650™		405231	100 µl

Brilliant Violet 711™			
Specificity	Clone	Cat. No.	Size
Human			
CD3	OKT3	317327	25 tests
CD4	OKT4	317439	25 tests
CD8a	RPA-T8	301043	25 tests
CD11c	3.9	301629 301630	25 tests 100 tests
CD14	M5E2	301837	25 tests
CD16	3G8	302043	25 tests
CD19	HIB19	302245 302246	25 tests 100 tests
CD25	BC96	302635	25 tests
CD27	O323	302833	25 tests
CD38	HIT2	303527	25 tests
CD45RA	HI100	304137	25 tests
CD45RO	UCHL1	304235 304236	25 tests 100 tests
CD56 (NCAM)	HCD56	318335 318336	25 tests 100 tests

Brilliant Violet 711™			
Specificity	Clone	Cat. No.	Size
Human			
CD127 (IL-7Rα)	A019D5	351327	25 tests
CD197 (CCR7)	G043H7	353227	25 tests
CD279 (PD-1)	EH12.2H7	329927	25 tests
HLA-DR	L243	307643	25 tests
IFN-γ	4S.B3	502539	25 tests
IL-17A	BL168	512327	25 tests
Ki-67	Ki-67	350515	25 tests
T-bet	4B10	644819	125 µl

Brilliant Violet 711™			
Specificity	Clone	Cat. No.	Size
Mouse			
CD4	RM4-5	100549 100550	125 µl 500 µl
CD8a	53-6.7	100747 100748	125 µl 500 µl
CD11b	M1/70	101241	125 µl
IFN-γ	XMG1.2	505835	125 µl
Ly-6A/E (Sca-1)	D7	108131	125 µl
T-bet	4B10	644819	125 µl

Brilliant Violet 711™			
	Clone	Cat. No.	Size
Isotype Controls			
Mouse IgG1, κ	MOPC-21	400167	25 tests

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Brilliant Violet™ Products Available

Brilliant Violet 785™			
Specificity	Clone	Cat. No.	Size
Human			
CD3	OKT3	317329	25 tests
CD4	OKT4	317441	25 tests
CD8a	RPA-T8	301045	25 tests
CD14	M5E2	301839	25 tests
CD16	3G8	302045	25 tests
CD19	H1B19	302239	25 tests
CD25	BC96	302637	25 tests
CD27	O323	302831	25 tests
CD45RA	HI100	304139	25 tests
CD45RO	UCHL1	304233	25 tests
CD127 (IL-7Ra)	A019D5	351329	25 tests
CD196 (CCR6)	G034E3	353421	25 tests
CD197 (CCR7)	G043H7	353229	25 tests
CD279 (PD-1)	EH12.2H7	329929	25 tests
HLA-DR	L243	307641	25 tests
IFN-γ	4S.B3	502541	25 tests

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Brilliant Violet 785™			
Specificity	Clone	Cat. No.	Size
Mouse			
CD3	17A2	100231	125 µl
CD4	RM4-5	100551	125 µl
CD8a	53-6.7	100749	125 µl
CD11c	N418	117335	125 µl
CD19	6D5	115543	125 µl
CD44	IM7	103041	125 µl
CD45R/B220	RA3-6B2	103245	125 µl
IFN-γ	XMG1.2	505837	125 µl

Brilliant Violet 785™			
	Clone	Cat. No.	Size
Isotype Controls			
Mouse IgG1, κ	MOPC-21	400169	25 tests
Rat IgG2a, κ	RTK2758	400545	125 µl
Rat IgG2b, κ	RTK4530	400647	125 µl

BioLegend Brilliant Violet™ Publications

- Beres, A.J., et al. 2012. *J. Immunol.* 189:464.
- Kinsey, G.R., et al. 2012. *J. Am. Soc. Nephrol.* 23: 1528.
- Xu, Y., et al. 2012. *J. Immunol.* 188:4113.
- Chattopadhyay, P.K., et al. 2012. *Cytometry A*. 81: 456.
- Chattopadhyay, P.K., and Roederer, M. 2012. *Methods* 57: 251.
- Nijnik, A., et al. 2012. *Blood* 119:1370.
- Soghoian, D.Z., et al. 2012. *Sci. Transl. Med.* 4:123ra25.
- Lemaître, F., et al. 2013. *J. Immunol.* 191: 1578.
- Covens, K., et al. 2013. *Blood* 121:5176.
- Dolfi, D.V., et al. 2013. *J. Leukoc. Biol.* 93: 825.
- de Silve, T.I., et al. 2013. *Blood* 121: 4330.
- Lawrence, T.M., et al. 2013. *J. Virol.* 87: 5848.
- Reading, J.L., et al. 2013. *J. Immunol.* 190: 4542.
- Xu, Y., et al. 2013. *J. Virol.* 87: 3760.
- DeFuria, J., et al. 2013. *PNAS* 110: 5133.
- Molofsky, A.B., et al. 2013. *J. Exp. Med.* 210: 535.
- Kim, G., et al. 2013. *J. Immunol.* 190: 1510.
- Leeansyah, E., et al. 2013. *Blood* 121: 1124.
- Takahashi, H., et al. 2012. *Stem Cell Trans. Med.* 1: 803.
- Lugli, E., et al. 2012. *Nature Protocols* 8: 33.
- Alexander, J.J., et al. 2012. *Kidney Intl.* 82: 961.
- Gatto, D., et al. 2013. *Nature Immunol.* 14: 446.
- Sage, P.T., et al. 2012. *Nature Immunol.* 14: 152.
- Baumjohann, D., et al. 2013. *Nature Immunol.* 14: 840.
- Laurenti, E., et al. 2013. *Nature Immunol.* 14: 756.
- De Keersmaecker, B., et al. 2012. *J. Virol.* 86: 9351.



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