Vio® Dyes technical specifications

Bright dyes for multicolor flow cytometry

Vio® Dyes represent a family of fluorochromes for flow cytometry and fluorescence microscopy developed by Miltenyi Biotec. VioBlue®, VioGreen™, VioBright™ FITC, PE-Vio® 615, PE-Vio® 770, PerCP-Vio® 700, Vio® 515, VioBright™ 515, APC-Vio® 770 are characterized by high fluorescence intensities and low spillover, making them an ideal choice for multicolor applications. Combined with traditional fluorochromes, such as FITC, PE, PerCP, and APC, the new Vio Dyes expand Miltenyi Biotec's antibody offering and allow researchers a greater selection of antibodies for multiparameter cell analysis.

Fluorochrome-conjugated MACS® Antibodies are perfectly suited for identification and enumeration of human, mouse, rat, or non-human primate cells.
Visit **www.macsantibodies.com** for a complete product list of MACS Antibodies, Detection Kits, Cytokine Secretion Assays, and Control Cocktails.

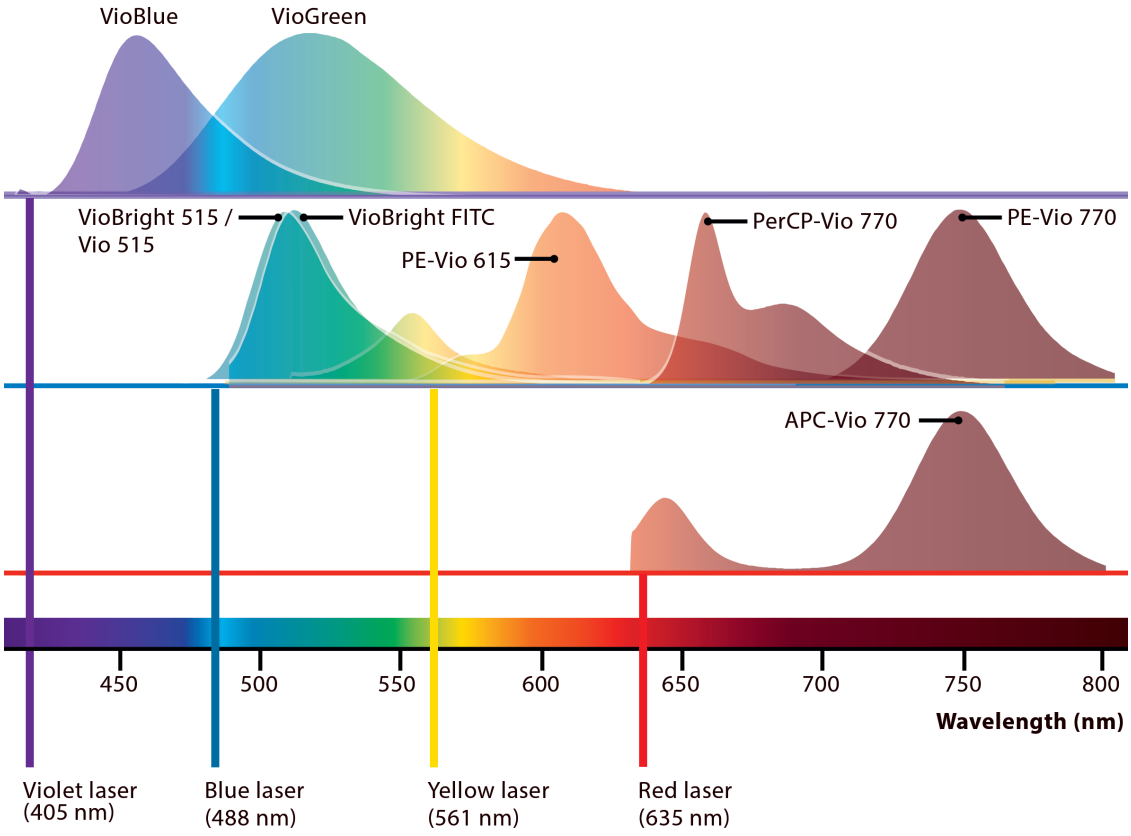

Figure 1: Emission spectra of Vio Dyes: VioBlue, VioBright 515, Vio 515, VioGreen, VioBright FITC, PE-Vio 615, PerCP-Vio 700, PE-Vio 770 and APC-Vio 770

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Overview of flurochromes available from Miltenyi Biotec

Fluorochrome	Excitation laser (nm)	Ex_{max} (nm)	Em_{max} (nm)	MACSQuant Analyzer		MACSQuant VYB	
				Channel	Filter (nm)	Channel	Filter (nm)
VioBlue	405	400	452	V1	450/50	V1	450/50
VioGreen	405	388	520	V2	525/50	V2	525/50
VioBright 515	488	488	514	B1	525/50	B1	525/50
Vio 515	488	488	514	B1	525/50	B1	525/50
VioBright FITC	488	496	522	B1	525/50	B1	525/50
FITC	488	495	520	B1	525/50	B1	525/50
PE	488 or 561	565	578	B2	585/40	Y1	586/15
PE-Vio 615	488 or 561	565	619	B3	655–730	Y2	615/20
PerCP	488	482	675	B3	655–730	N/A	N/A
PerCP-Vio 700	488	482	704	B3	655–730	N/A	N/A
PE-Vio 770	488 or 561	565	775	B4	750 LP	Y4	750 LP
APC	561 or 635	652	660	R1	655–730	Y3	661/20
APC-Vio 770	561 or 635	652	775	R2	750 LP	Y4	750 LP

Table 1: Fluorochrome specifications.

Miltenyi Biotec dyes	Other dyes
VioBlue®	Alexa Fluor® 405, BD™ Horizon™, V450, BV 421™, Calcein Violet 450 AM, Cascade Blue®, DAPI, Vybrant®, DyeCycle™ Violet, eBFP, eFluor® 450, Hoechst Dyes, Pacific Blue™, Zombie Violet™
VioGreen™	Alexa Fluor® 430, AmCyan, BD™ Horizon™ V500, BV510™, Cascade Yellow™, Krome Orange™, Pacific Orange™, Qdot® 525 Zombie Aqua™
FITC, VioBright™ FITC, Vio® 515, VioBright™ 515	Alexa Fluor® 488, Calcein AM, DyLight® 488, CFSE, GFP, SYTOX® Green, Vybrant® DyeCycle™ Green, YFP, Zombie Green™, BD Horizon™ Brilliant Blue 515
PE	Cy™3, Vybrant® DyeCycle™ Orange
PerCP, PerCP-Vio® 700	PE-Cy™5, PE-Cy™5.5, Per-Cy™5.5, Per-CP-eFluor® 710, Propidium iodide, 7-AAD
PE-Vio® 615	ECD, PE-Texas Red®, PE-CF594, PE/Dazzle™ 594, PE-eFluor® 610
PE-Vio® 770	PE-Alexa Fluor® 750, PE-Cy™ 7
APC	Alexa Fluor® 647, Alexa Fluor® 700, APC-Alexa Fluor® 700, Cy™5, DRAQ5®, eFluor® 660
APC-Vio® 770	APC-Alexa Fluor® 750, APC-Cy™7, APC-eFluor™ 780, APC-H7, Zombie NIR™

Table 2: Available Vio Dye fluorochromes.

Support Material

- Fluorescence spectra overview poster
- Fluorochromes and instruments poster
- Fluorochrome brightness index
- Lot-to-lot consistency data
- Staining enhancement products
- Viability dyes

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Violet laser dyes

VioBlue® and VioGreen™ Fluorochromes are a novel range of violet laser excited dyes developed exclusively by Miltenyi Biotec. Designed to maximize the potential of a flow cytometer's violet laser, they not only show superior optical performance compared with many other 405 nm excited fluorochromes, but also significant advances in brightness, signal-to-noise ratios, and intra-laser compensation requirements. In addition, neither fluorochrome exhibits significant levels of photoinduced degradation, and can consequently be used for many different applications, such as fluorescent microscopy.

VioBlue® Dye

- Coumarin-based dye with excitation and emission wavelengths of 405 nm and 455 nm, respectively
- Superior alternative to Pacific Blue, Alexa Fluor 405, or BD Horizon V450
- Multiplexing of VioBlue with other fluorochromes is easily possible, adding to the variety of marker combinations for multiparameter cell analysis
- Combined use with the VioGreen Dye

Fluorochrome	Absorption max. (nm)	Emission max (nm)
VioBlue	400	455
Pacific Blue	401	452
Cascade Blue	401	419
Alexa Fluor 405	401	421
eFluor 450	405	450
BD Horizon V450	404	448
BD Horizon Brilliant™ Violet 421	407	421

Table 3: Absorption and emission maximums of fluorochromes related to VioBlue.

Laser and filter compatibility

With a maximum absorption and emission at 400 nm and 455 nm respectively, VioBlue Fluorochromes are fully compatible with standard filter sets from all major flow cytometry hardware providers, giving researchers the flexibility to use the VioBlue Dye with existing laboratory platforms (figure 2).

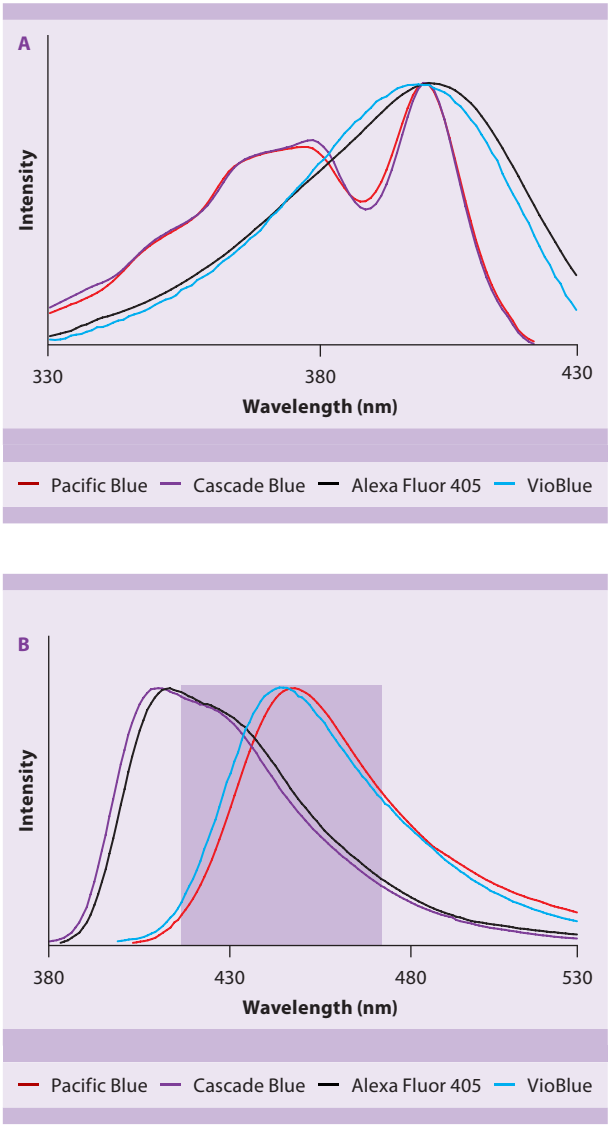

Figure 2: Absorption (A) and emission (B) spectra of VioBlue compared to Pacific Blue, Cascade Blue, and Alexa Fluor 405. The blue box represents the 450/50 nm filter.

Enhanced brightness

Human peripheral blood mononuclear cells (PBMC) and mouse splenocyte (MS) cells stained with VioBlue® exhibit excellent discrimination between positively and negatively stained populations over a wide range of antibody specificities (figure 3). Furthermore, VioBlue provides a superior alternative to many spectrally similar conjugates for the V1 channel, further increasing the options of multicolor analysis (figure 4).

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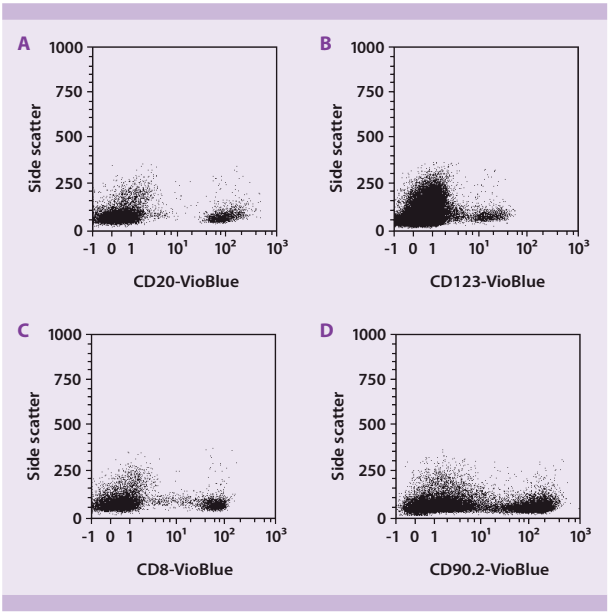

Figure 3: Human peripheral blood mononuclear cells (PBMC) or mouse splenocyte (MS) cells were stained with CD20-VioBlue (A; PBMCs), the rare cell marker CD123-VioBlue (B; PBMCs), CD8-VioBlue (C; PBMCs), or CD90.2-VioBlue (D; MS) and analyzed by flow cytometry using the MACSQuant Analyzer.

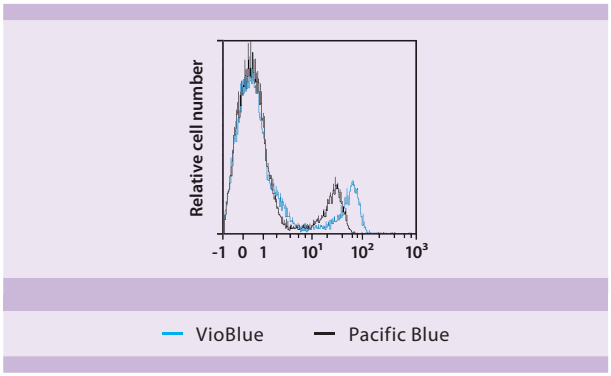

Figure 4: Fluorescence intensity of cells labeled with CD14 antibodies conjugated to either VioBlue or Pacific Blue.

Decreased spillover

VioBlue® Dyes exhibit minimal spillover into the V2 channel, making them perfect candidates for multicolor panels, which utilize both violet channels. Furthermore, VioBlue is negligibly excited by the 488 nm laser, and thus requires no compensation between the V1 and B1 channels.

High stability during fixation

It is of crucial importance for a conjugate to retain its fluorescent properties after fixation, in order to allow researchers to maximize the use of biological samples. The VioBlue Dye has a very high stability after fixation with paraformaldehyde (figure 5), equal to or exceeding many other spectrally similar fluorochromes.

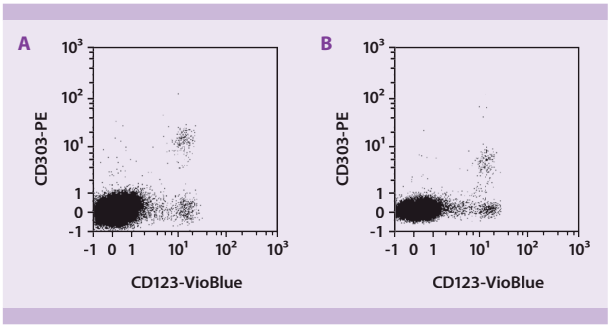

Figure 5: CD123-VioBlue staining before (A) and after (B) fixation with 3.7% paraformaldehyde, indicating a minimal decrease in fluorescence after fixation.

Lot-to-lot variation

Reproducible lot-to-lot antibody performance ensures the integrity of long-term studies, and is crucial in comparing experimental results over a period of time. All MACS® Antibodies are vigorously tested to ensure consistent quality, thus eliminating the need for repeat testing arising from reagent degradation or failure (figure 6).

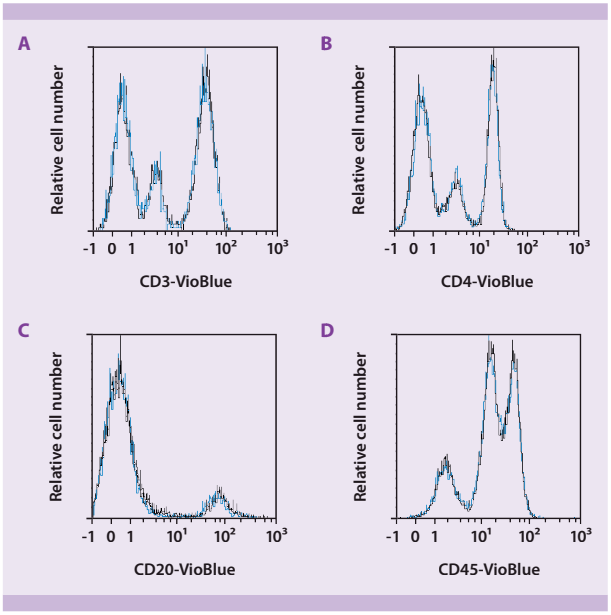

Figure 6: Lot-to-lot variation for different lots of CD3 (A), CD4 (B), CD20 (C), and CD45 (D) antibodies conjugated to VioBlue. Black and blue lines represent different antibody lots.

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VioGreen™ Dye

- Large Stokes shift fluorochrome, emitting strong fluorescence at 520 nm upon excitation at 405 nm
- Significantly increased mean fluorescence intensities and higher stain indices over Pacific Orange, Krome Orange, and BD Horizon V500
- Non-protein fluorochrome
- Suitable for ethanol fixation
- Combined use with the VioBlue® Dye
- Perfectly suited for multiparameter cell analysis using all current flow cytometers

Fluorochrome	Absorption max. (nm)	Emission max. (nm)
VioGreen	388	520
Pacific Orange	400	551
Krome Orange	398	528
AmCyan	457	491
Horizon V500	415	500
BD Horizon Brilliant™ 510	405	510

Table 4: Absorption and emission maximums of fluorochromes comparable to comparable to VioGreen™.

Laser and filter compatibility

With a standard 525/50 filter set, the VioGreen™ Dye exhibits a better spectral profile than Pacific Orange or AmCyan (figure 7).

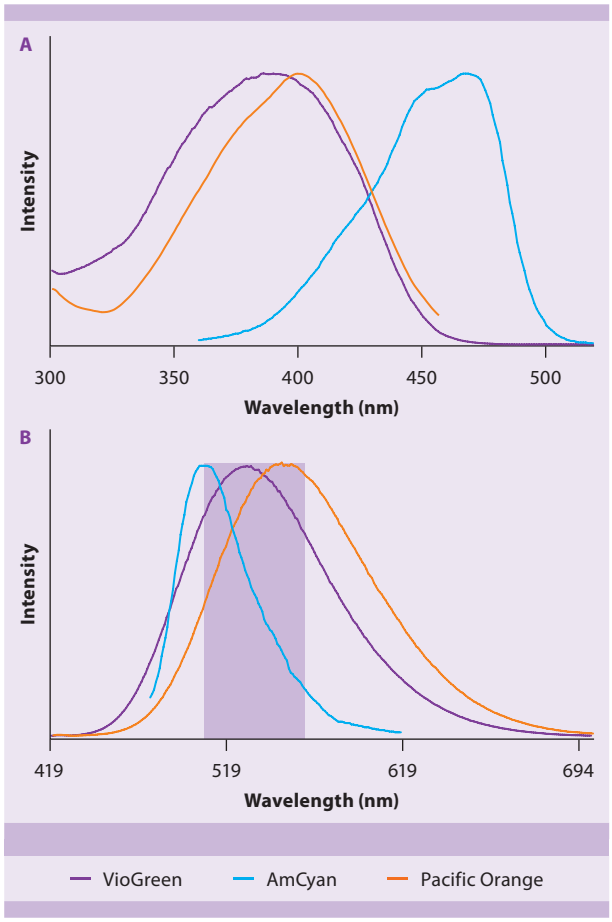

Figure 7: Absorption and emission spectra of VioGreen.

Enhanced brightness

Like most dyes designed for the violet laser, VioGreen shows a lower fluorescence intensity compared to other well-established fluorochomes, such as PE. However, specific human and mouse cellular populations are easily distinguishable between positively and negatively labeled cells (figure 8). In addition, many VioGreen Dyes exhibit brighter fluorescence compared to spectrally similar conjugates, such as Pacific Orange (figure 9 and table 5), AmCyan, and Horizon V500, when measured by mean fluorescence intensity (MFI) or stain index (normalized signal-to-noise ratio).

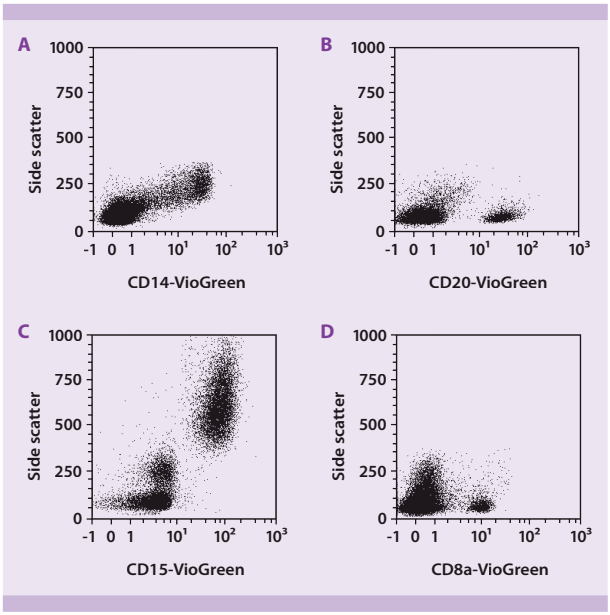

Figure 8: Human peripheral blood mononuclear cells (PBMC) or mouse splenocyte (MS) cells were stained with CD14-VioGreen (A; PBMCs), CD20-VioGreen (B; PBMCs), CD15-VioGreen (C; PBMCs), or CD8a-VioGreen (D; MS) and analyzed by flow cytometry using the MACSQuant® Analyzer.

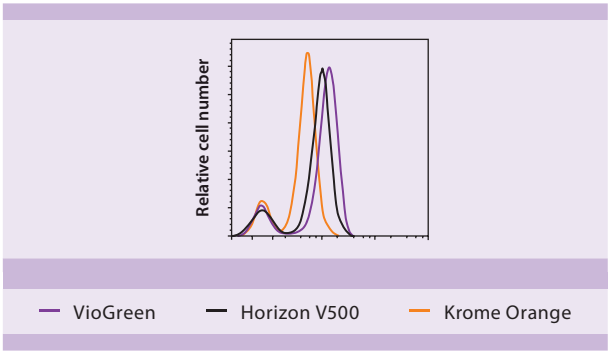

Figure 9: Analysis of human PBMCs using CD3 antibodies conjugated to either VioGreen, Horizon V500, or Krome Orange.

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Conjugate	Clone	MFI	Stain index	Percent spillover	
				V1	B1
CD3-VioGreen	BW264/56	13	13	5.5	0.5
CD3-Horizon V500	UCHT1	11	9	8.0	1.5
CD3-Krome Orange	UCHT1	6	7	3.0	1.0
CD8-VioGreen	BW135/80	16	17	6.0	0.2
CD8-Pacific Orange	BW135/80	10	11	3.0	0.5

Table 5: MFI, stain indices, and % spillover of CD3-VioGreen, CD3-Horizon V500, CD3-Krome Orange, CD8-VioGreen, and CD8-Pacific Orange.

High stability during paraformaldehyde and ethanol fixation

VioGreen™ Dyes exhibit strong photostability during fixation, with only very minimal photodegradation, thus highlighting VioGreen's suitability as a fluorochrome for use in studies that require fixation (figure 10). It is also suitable for ethanol fixation.

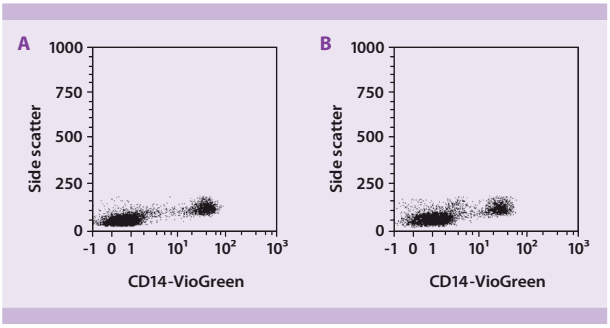

Figure 10: CD14-VioGreen staining before (A) and after (B) paraformaldehyde fixation, indicating a minimal decrease in fluorescence after fixation.

Blue laser dyes

VioBright™ FITC, VioBright 515 and Vio® 515 Dyes

VioBright™ FITC, VioBright 515 and Vio® 515 are revolutionary blue laser (488 nm) excitable dyes, developed exclusively by Miltenyi Biotec. The innovative VioBright technology allows for an increased number of fluorochrome molecules per antibody, as compared to conventional conjugation. This results in dyes that emit a signal detected in the standard FITC channel, but with brightness levels similar to PE. These blue laser dyes expand the dimensions of multicolor flow analysis and provide a bright alternative for confident detection of rare cells, dim, and uncharacterized markers.

Dye	Features	Application
VioBright FITC (1st generation VioBright dye)	• High brightness	Detection of extracellular markers
VioBright 515 (2nd generation VioBright dye)	• Very high brightness • Low spill over • High fixation- & photostability	Detection of extracellular markers
Vio 515 (Organic dye)	• Moderate brightness, • Low spill over • High fixation- & photostability	Detection of intracellular markers

Table 6: Features and application specifications for VioBright FITC, VioBright 515 and Vio 515 in comparison.

Laser and filter compatibility

Excitable by the blue laser (488 nm), VioBright 515 and Vio 515 display peak excitation and emission at 488 nm and 514 nm, respectively (figure 11 and table 7). VioBright FITC is excited at 496 nm with a peak emission at 522 nm. The high intensity fluorescent signals can be detected with a standard FITC filter such as 525/50 on MACSQuant Instruments. Thus, no change in the filter or cytometer is required. Along with PE, the VioBright FITC, VioBright 515 and Vio 515 dyes enhance the potential of the blue laser, one of the most common laser lines available for single laser to multi-laser instruments.

Fluorochrome	Absorption max. (nm)	Emission max. (nm)
VioBright 515	488	514
Vio 515	488	514
VioBright FITC	496	522
FITC	495	520
Alexa Fluor 488	495	519
BD Horizon Brilliant Blue 515	490	515

Table 7: Absorption and emission maximums of fluorochromes comparable to VioBright FITC, VioBright 515 and Vio 515

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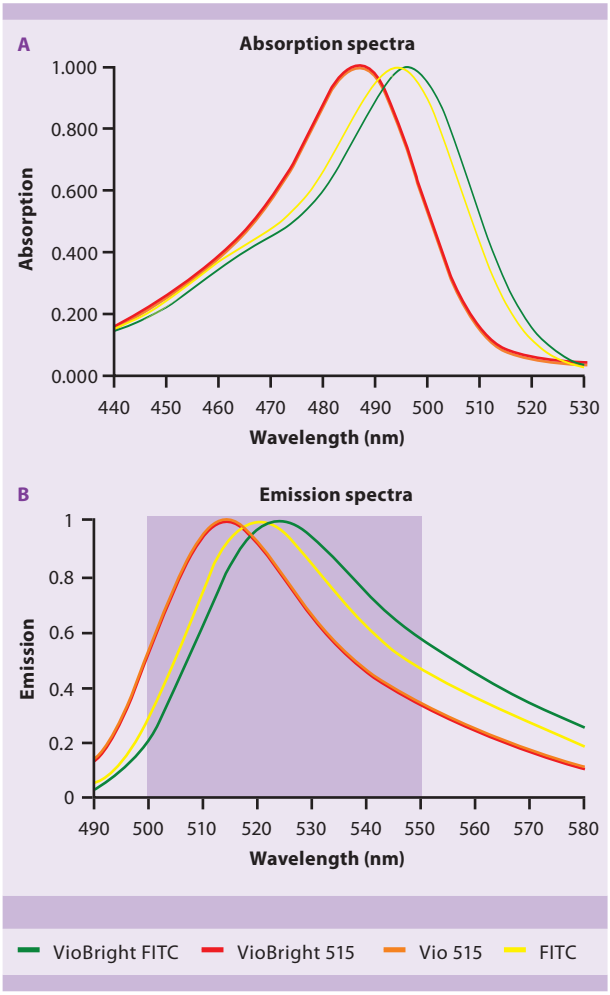

Figure 11: Absorption (A) and emission (B) spectra of VioBright™ FITC, VioBright 515 and Vio 515 compared to FITC.

Enhanced brightness

VioBright™ FITC, VioBright 515 and Vio® 515 are superior dyes for detection of low-expressed markers, as shown in figure 12 and 13. With stain indices (SI) and mean fluorescent intensities similar to PE, staining with these novel dyes allows for an excellent resolution of positive and negative populations.

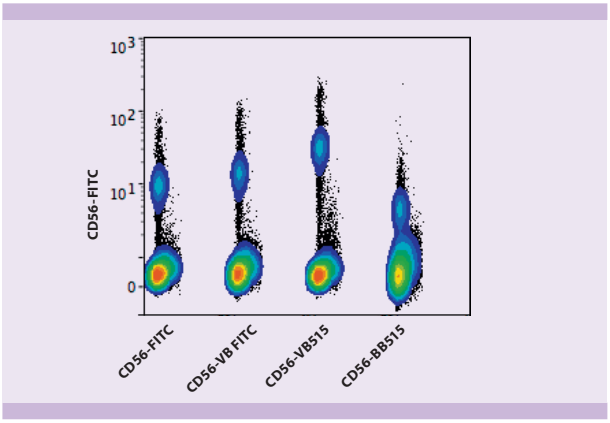

Figure 12: Human PBMCs were stained using CD56 antibodies conjugated to FITC, VioBright FITC, VioBright 515, or BB515 and analyzed by flow cytometry using the MACSQuant® 10 Analyzer.

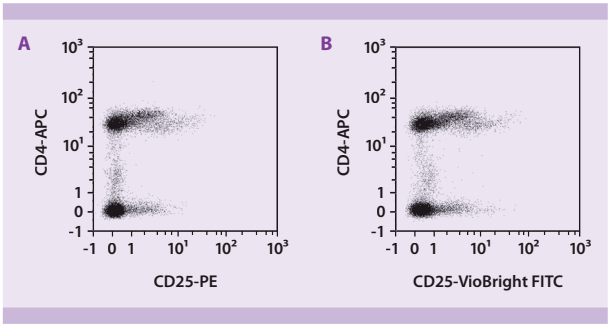

Figure 13: Human PBMCs were stained with CD25 antibodies conjugated to PE or VioBright FITC, as well as with CD4-APC, and analyzed by flow cytometry using the MACSQuant Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

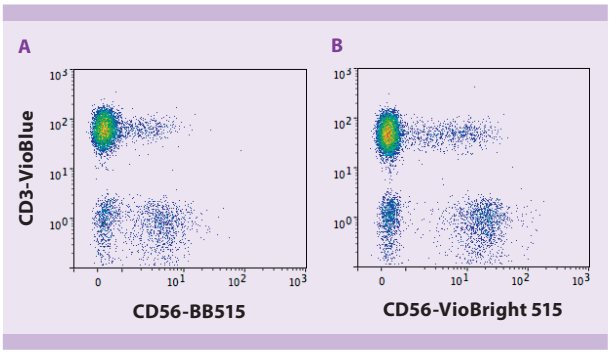

Figure 14: Human whole blood was stained with CD3-VioBlue® and CD56-VioBright™ 515 or CD56-BB515, respectively.

Reliable analysis of rare cells requires specific identification of low frequency cellular subset and clear target population resolution. Thus, highly specific antibodies together with bright conjugates are a prerequisite for detection of such low frequency populations. As demonstrated in figures 11–14, VioBright FITC and VioBright 515 represent bright dye alternatives for optimal detection of your subpopulation of interest and detailed phenotypic analysis.

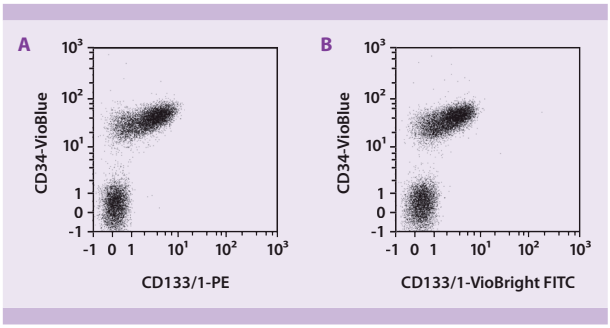

Figure 15: CD34⁺ enriched human PBMCs were stained with CD133/1 antibodies conjugated to PE or VioBright FITC as well as with CD34-VioBlue and analyzed by flow cytometry using the MACSQuant Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

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Conjugate	MFI		Stain index	
	VioBright FITC	PE	VioBright FITC	PE
CD25	3.3	2.0	5.0	4.0
CD335	4.8	4.0	8.5	11
CD133/1	3.5	4.7	4.7	4.7

Table 8: MFI and stain indices of CD25 (clone 4E3), CD335 (clone 9E2), and CD133/1 (clone AC133) antibodies conjugated to either VioBright FITC or PE.

Spill over

Compared to standard FITC, VioBright 515 and Vio® 515 dyes display reduced spill over into the PE channel (table 8). In contrast, VioBright FITC has a slightly higher spill over into the PE channel. Thus, with nominal change in compensation settings, these innovative dyes offer the benefit of a brighter staining.

Sample	Conjugate	MFI	Stain index	MACSQuant Analyzer 10 Compensation in channel B2 (%)	MACSQuant VYB Compensation in channel Y1 (%)
A	CD4-VioBright FITC	66	106	8.5	0
A	CD4-FITC	29	49	6.5	0
A	CD4-PE	58	126	–	–
B	CD4-VioBright FITC	52	81	9.3	0
B	CD4-FITC	20	35	7.0	0
B	CD4-PE	39	73	–	–

Table 9: Compensation requirements in PE channel (B2) of CD4 (clone VIT4) antibodies conjugated to VioBright FITC, FITC, or PE.

Conjugate	Clone	MFI	Stain index	MACS Quant Compensation in Channel B2 [%]
CD56-FITC	REA196	10.6	18.3	6.6
CD56-VioBright FITC	AF12	15.8	22.6	8.6
CD-VioBright 515	REA196	31.9	56.3	4.2
CD56-BB515	B159	4.9	4.4	6.2

Table 10: Mean fluorescent intensities (MFI) and stain indices of anti-CD56 antibodies conjugated to FITC, VioBright 515, and BB515.

Fixation stability

Bright fluorochrome conjugates, such as PE and APC, are often sensitive to fixatives like Methanol. For a successful flow cytomeric analysis, which often involves staining of intracellular markers, stability of the fluorochrome conjugates is critical. VioBright 515 conjugates show excellent stability to methanol- and paraformaldehyde-based fixatives with only little compromise in brightness (figure 16).

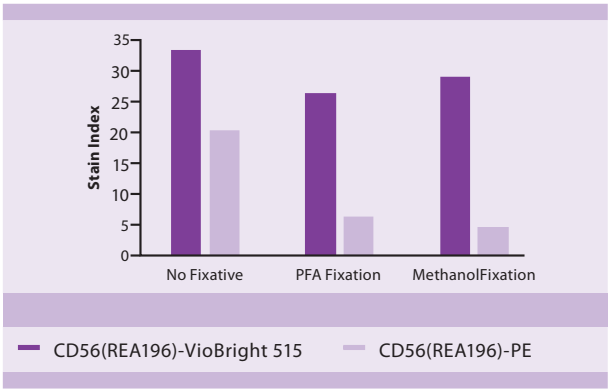

Figure 16: Human PBMCs were stained using CD56 antibodies (clone REA196) conjugated to VioBright 515 and PE. In addition, cells were analyzed before and after fixation using paraformaldehyde and 90% methanol. Stained cells were analyzed by flow cytometry using the MACSQuant® 10 Analyzer

Photo stability

The stability of VioBright 515 upon exposure to confocal laser light was analyzed at different time points on a Zeiss LSM710 confocal microscope. Significantly higher mean fluorescence intensity (MFI) compared to FITC conjugate, were demonstrated for these time points (figure 17). VioBright 515 shows comparable stability to Alexa Fluor 488 conjugated antibodies indicating the excellent suitability for immunofluorescence microscopy.

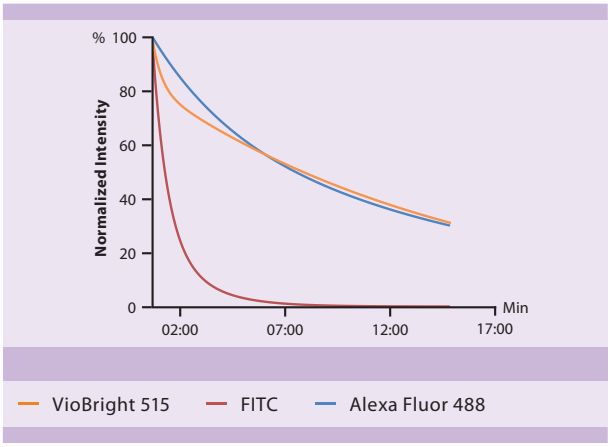

Figure 17: Human PBMCs were stained with fluorochrome-conjugated CD4 antibodies. Stained cell samples were fixed with p-formaldehyde for 20 min and transferred to a 96-well plate for analysis. The plotted curve is normalized over multiple time points.

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Blue laser tandem conjugates

PE-Vio® 615 Tandem Conjugate

PE-Vio® 615 is a tandem dye with PE as the donor dye and Vio® 615 as the acceptor dye. This tandem dye is optimized for efficient donor-to-acceptor dye energy transfer, high fluorescent intensity, and low spillover into the donor dye detection channel. Designed to be a superior alternative to ECD, PE-Texas Red®, PE-efluor® 610, PE-CF594 and PE/Dazzle™ 594, this Vio Dye expands the options for flexible multicolor panel design and provides a bright dye for confident detection of dim and rare markers.

- Optimal excitation with blue (488 nm), green (532 nm), and yellow green (561 nm) laser lines for maximum flexibility.
- Excellent brightness for confident detection of dim, rare, and uncharacterized markers.
- Extensive portfolio of PE-Vio 615 dye conjugated to recombinantly engineered REAfinity Antibodies for higher reproducibility.

Fluorochrome	Absorption max (nm)	Emission max (nm)
PE-Vio 615	565	619
ECD, PE-Texas Red	565	613
PE-efluor 610	565	606
PE-CF594	565	614
PE/Dazzle 594	566	612

Table 11: Absorption and emission maximums of fluorochromes comparable to PE-Vio 615.

Laser and filter compatibility

The PE molecule shows peak absorption at two wavelengths, 496 nm and 565 nm, respectively. This allows for optimal excitation of PE-containing tandem dyes such as PE-Vio 615 using blue, green, and yellow green laser lines (488-561 nm). This enables the utilization of the maximum potential of your instrument with flexible choice of laser lines (figure 19). As depicted in figure 15, the extent of absorption at 565 nm is greater than 496 nm nm and thus, the maximum excitation of PE-Vio 615 can be achieved with instruments such the MACSQuant VYB, which uses yellow laser lines for excitation of PE and all PE containing tandem dyes. The emission signal can be detected using typical filters design to detect PE-Texas Red, such as 615/20 nm.

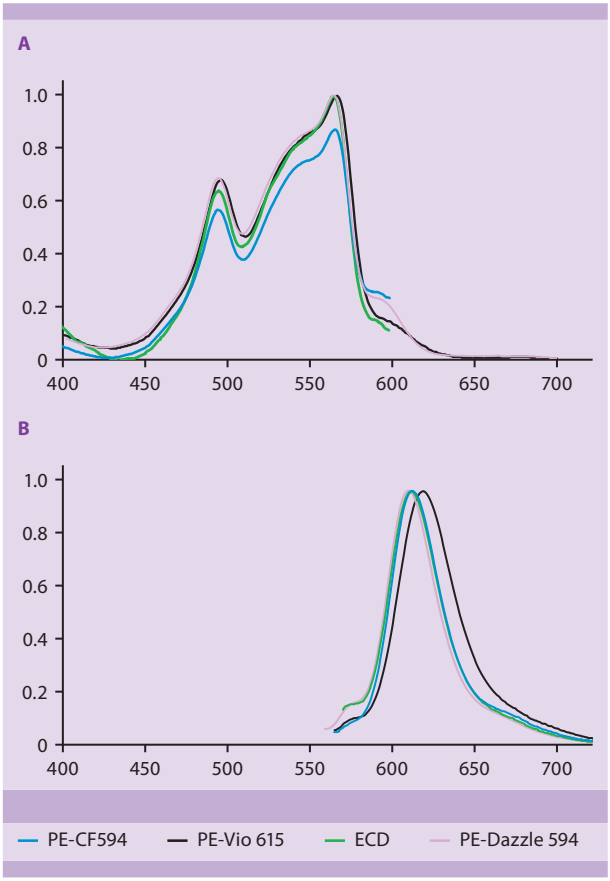

Figure 18: Absorption (A) and emission (B) spectra of PE-Vio 615 and comparable fluorochromes.

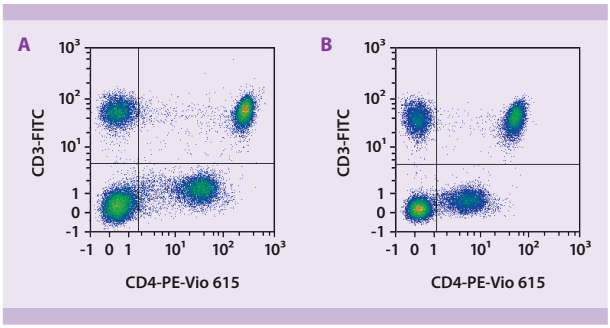

Figure 19: Human PBMCs were stained using CD4 antibodies (clone VIT4) conjugated to PE-Vio615 followed by analysis using MACSQuant VYB (A) and MACSQuant Analyzer (B).

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Enhanced brightness

PE-Vio® 615 is designed to offer a bright alternative to comparable dyes such as PE-efluor 610, PE-CF594, ECD, and PE/Dazzle 594 (figure 20). PE-Vio 615 not only allows for high fluorescent intensities but also excellent separation of positive population for higher stain indices (figure 21). This property of PE-Vio 615 makes it an excellent dye for analysis of dim and difficult to characterize markers (figure 22).

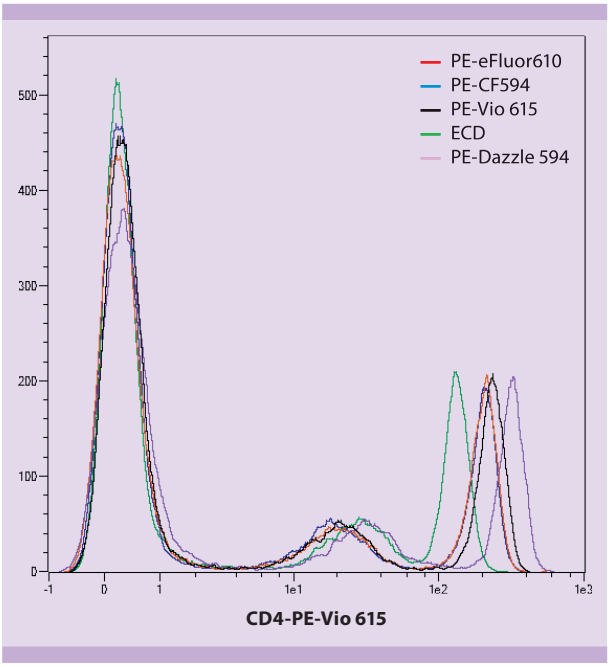

Figure 20: Human PBMCs were stained using CD4 antibodies conjugated to PE-eFluor610, PE-CF594, PE-Vio615, ECD, PE-Dazzle594 and analyzed by flow cytometry using the MACSQuant VYB.

Conjugate	Clone	MFI	Stain index	MACS Quant VYB Compensation in channel Y1 [%]
CD4-PE-Vio 615	Vit-4.3	236	309	3.0
CD4-ECD	SFCI12T4D11	133	185	3.3
CD4-PE-CF594	RPA-T4	201	295	2.6
CD4-PE-eFluor610	RPA-T4	202	259	5.5
CD4-PE/Dazzle594	RPA-T4	316	287	3.9

Table 12: MFI and stain indices of CD4 antibodies conjugated to PE-Vio 615, ECD, PE-CF594, PE-eFluor610, PE/Dazzle594.

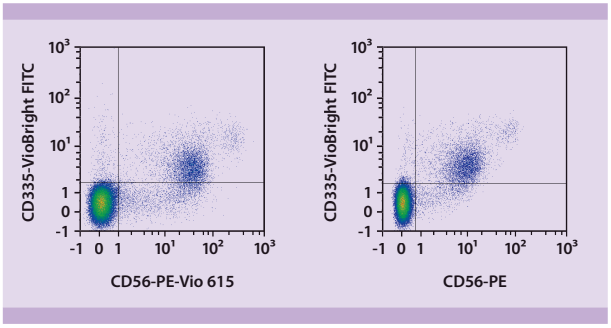

Figure 21: Human PBMCs were stained using CD56 antibodies (clone REA196) conjugated to either PE-Vio 615 or PE and CD335-VioBright FITC. Stained cells were then analyzed by flow cytometry using the MACSQuant VYB.

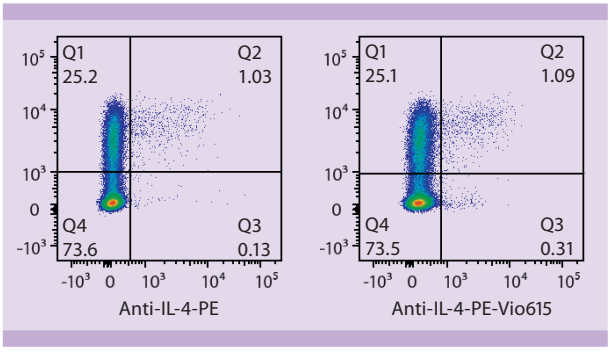

Figure 22: PBMCs were stimulated for 6 hours. After 2 hours, 1 µg/mL BrefeldinA was added for the last 4 hours. Cells were surface stained with CD4-FITC, fixed and permeabilized with the Inside Stain Kit (Miltenyi Biotec) and stained intracellularly with CD154-VioBlue and Anti-IL-4-PE or Anti-IL-4-PE-Vio615 (clone: 7A3-3). Cells were acquired on a BD Fortessa. Cells are gated on $CD4^+$ lymphocytes, numbers indicate frequency among $CD4^+$ cells.
Data courtesy: Petra Bacher, Clinic for Rheumatology and Clinical Immunology, Charité – University Medicine Berlin, Berlin, Germany

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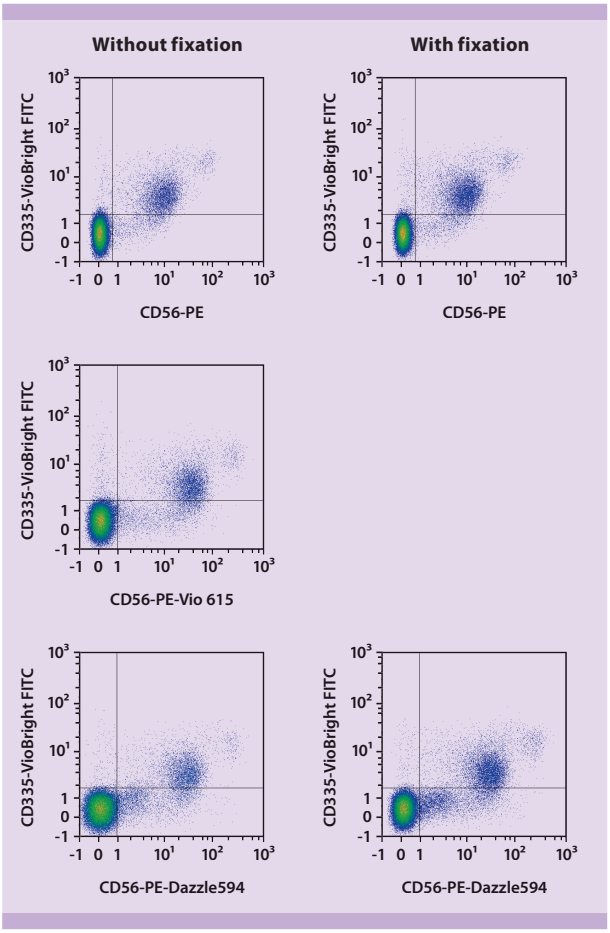
Fixation stability

Tandem conjugates are often sensitive to fixatives. Thus, for a successful flow cytomeric analysis, stability of tandem conjugates is critical. PE-Vio® 615 conjugate shows excellent stability to paraformaldehyde-based fixative without any increase in background signal (figure 23).



Conjugate	Clone	without fixation		with fixation	
		MFI	Stain index	MFI	Stain index
CD56-PE	REA196	12.0	33.2	11.9	32.7
CD56-PE-Vio 615	REA196	39.9	70.9	40.1	71.4
CD56-PE-Dazzle594	HCD56	30.5	42.2	31.5	54.0

Figure 23: Human PBMCs were stained using CD56 antibodies (clone REA196) conjugated to PE, PE-Vio615 or PE-Dazzle594. In addition, cells were stained with CD335-VioBright FITC and analyzed before and after fixation using paraformaldehyde. Stained cells were analyzed by flow cytometry using the MACSQuant VYB.

Photo stability

The stability of PE-Vio 615 upon exposure to ambient light (~850 Lux) was analyzed at different time points. Significantly higher mean fluorescence intensities (MFI) and stain indices (SI) for these time points, compared to commercially available alternatives, were also demonstrated (figure 23).

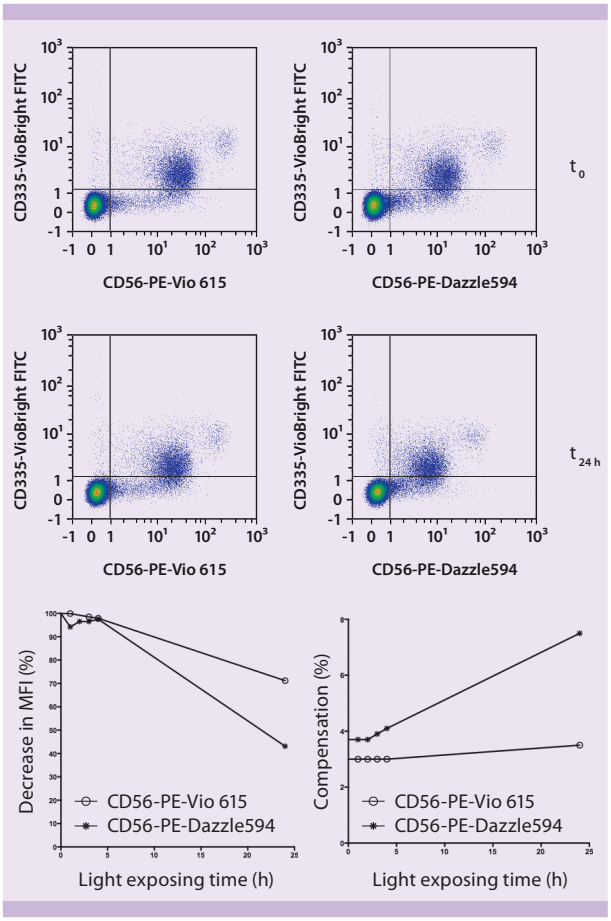


Figure 24: Human PBMCs were stained, using CD56 antibodies conjugated to PE-Vio615 (clone REA196) or PE-Dazzle594 (clone HCD56). Stained cells were then exposed to ambient light (~850 Lux) followed by analysis at different time points by flow cytometry using the MACSQuant VYB. Spill over in the Y1 channel, which is optimized for detection of PE signal was also analyzed after exposure to light.

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PerCP-Vio® 700 Tandem Conjugate

- Combines the peridinin chlorophyll protein (PerCP) and the new Vio® 700 dye
- Emits strong fluorescence at 655–730 upon blue laser excitation at 488 nm
- Suitable for the B3 channel of the MACSQuant Analyzer

Fluorochrome	Absorption max. (nm)	Emission max. (nm)
PerCP	482	675
PerCP-Vio 700	482	704
PerCP-Cy5.5	490	695

Table 13: Absorption and emission maximums of fluorochromes related to PerCP-Vio700.

Laser and filter compatibility

Using a standard 655–730 bandpass filter, PerCP-Vio 700 exhibits a very narrow fluorescence spectral profile, thus allowing the majority of light to be captured and retained (figure 25).

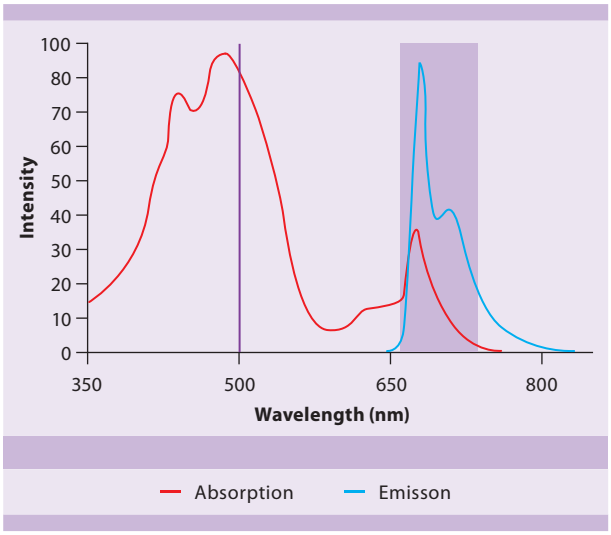

Figure 25: Absorption and emission spectra of PerCP-Vio 700. The blue box represents the 655–730 filter.

Enhanced brightness

Human peripheral blood mononuclear cells (PBMC) and mouse spenocyte (MS) cells were stained with PerCP-Vio 700, and consequently exhibited excellent separation between positive and negative populations over many different antibody specificities (figure 26).

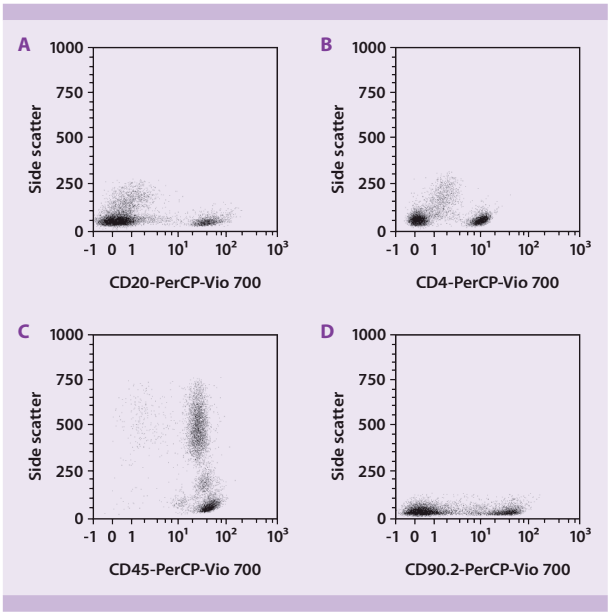

Figure 26: Human peripheral blood mononuclear cells (PBMC) or mouse spenocyte (MS) cells were stained with CD20-PerCP-Vio 700 (A; PBMCs), CD4-PerCP-Vio700 (B; PBMCs), CD45-PerCP-Vio 700 (C; PBMCs) or CD90.2-PerCP-Vio 700 (D; MS) and analyzed by flow cytometry using the MACSQuant Analyzer.

High stability during fixation

PerCP-Vio 700 shows excellent fixation stabilities, with only minimal decreases in fluorescence after fixation (figure 27).

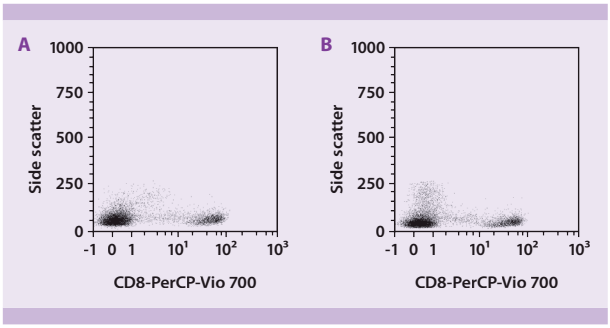

Figure 27: CD8-PerCP-Vio 700 before (A) and after (B) fixation with 3.7% paraformaldehyde, indicating a minimal decrease in fluorescence after fixation.

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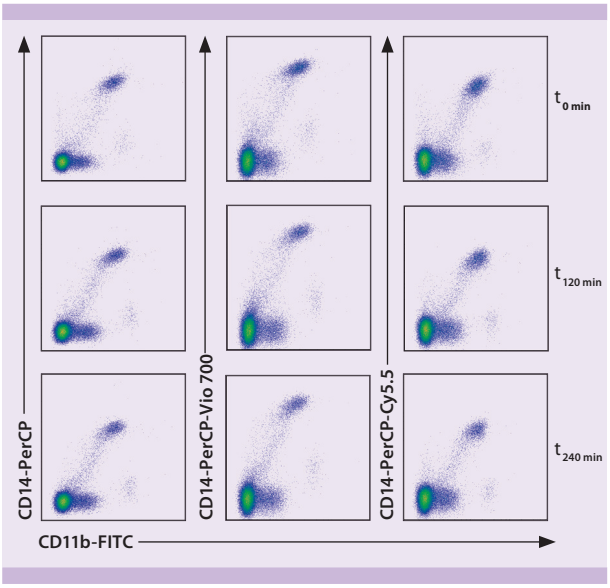
Photo-induced conjugate degradation

Analysis of the photo-induced degradation of CD14-PerCP-Vio 700 indicated no discernable changes after up to four hours of continuous exposure to ambient light (~850 Lux). Significantly higher mean fluorescence intensities (MFI) and stain indices (SI) for these time points, compare to commercially available alternatives (BD Biosciences), were also demonstrated (figure 28).



Marker	Clone	Stain index		
		$t_{0\ min}$	$t_{120\ min}$	$t_{240\ min}$
CD14-PerCP	TÜK4	53	44	36
CD14-PerCP-Vio 700	TÜK4	75	75	75
CD14-PerCP-Cy5.5	61D3	36	31	28

Figure 28: Photo-induced conjugate degradation of PerCP, PerCP-Vio 700, and PerCP-Cy5.5 with corresponding MFI and SI. Conjugates were exposed to ambient light for up to 4 hours, with negligible degradation rates.

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PE-Vio® 770 Tandem Conjugate

The PE-Vio® 770 Dye is a tandem conjugate, like PE-Cy™7, that exploits the principle of fluorescence-resonance-energy-transfer (FRET) allowing for large Stokes shifts between the absorbed energy of a fluorescence donor (PE) and the emission wavelength of a suitable acceptor (Vio 770) dye.

- Excitation with the blue (488 nm) or yellow (561 nm) laser, emission in the near-infrared region at 775 nm
- The combination of Vio 770 as the acceptor dye and an optimized chemistry have furnished a tandem dye that is characterized by a high fluorescence intensity, minimal spillover to adjacent detection channels, and low non-specific binding to non-target cells to meet the complexity of multiparameter cell analysis
- Higher MFI and stain index values, in addition to lower compensation settings when compared to PE-Cy7

Fluorochrome	Absorption max. (nm)	Emission max. (nm)
PE-Vio 770	496, 565	775
PE-Cy7	496, 565	785
PE-Alexa Fluor 750	496, 546	775

Table 14: Absorption and emission maximums of fluorochromes comparable to PE-Vio770.

Laser and filter compatibility

The tandem dyes PE-Vio 770, PE-Cy7, and PE-Alexa Fluor 750 all use PE as the donor molecule, showing absorption at 496 nm and to a greater extent at 565 nm. Consequently, flow cytometers, such as the MACSQuant VYB, equipped with a yellow 561 nm laser, provide maximum excitation to PE molecules, which in turn transfer this energy to the respective acceptor molecule. Few differences in the emission properties can be found between Vio 770, Cy7, and Alexa Fluor 750, making each tandem best suited to flow cytometry platforms using 750 LP filters (figure 21).

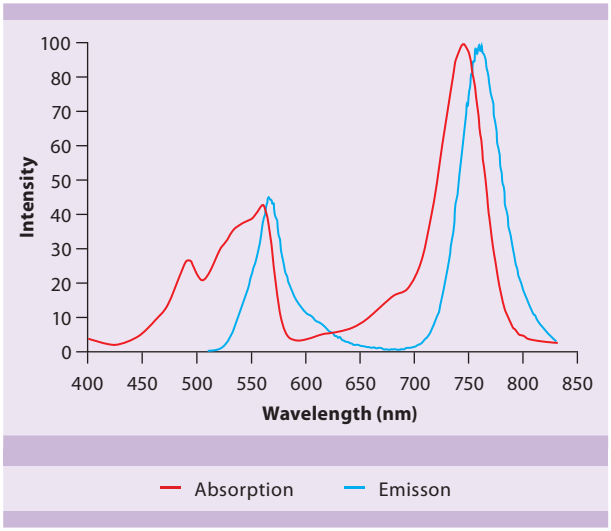

Figure 29: Absorption and emission spectra of PE-Vio 770.

Enhanced brightness

PE-Vio 770 provides the greatest fluorescence intensity of the Vio Dye family, with excellent separation of positively and negatively stained cells (figure 30). When compared to other spectrally similar tandem conjugates, such as PE-Cy7 (figure 31) or PE-Alexa Fluor 750, PE-Vio 770 exhibits significantly higher mean fluorescence intensities (MFI) and stain indices, and requires less compensation (table 14). These properties make PE-Vio 770 a far superior tandem conjugate compared to PE-Cy7 or PE-Alexa Fluor 750.

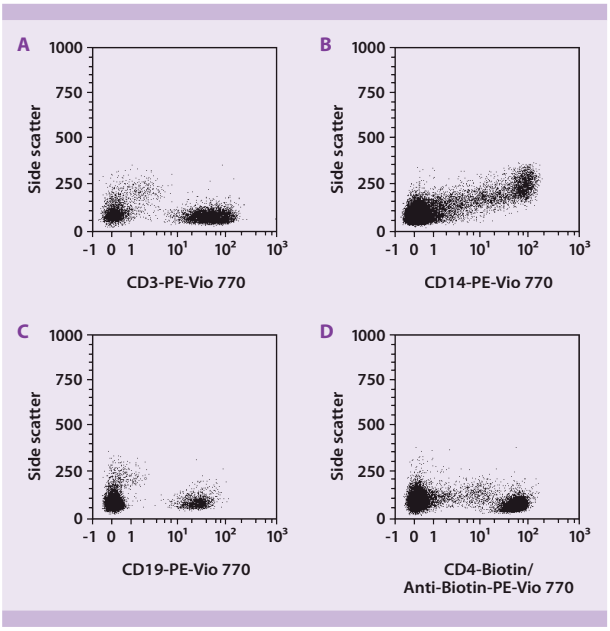

Figure 30: Human peripheral blood mononuclear cells (PBMC) or mouse splenocyte (MS) cells were stained with CD3-PE-Vio 770 (A; PBMCs), CD14-PE-Vio 770 (B; PBMCs), CD19-PE-Vio770 (C; PBMCs), or CD4-Biotin/Anti-Biotin-PE-Vio 770 (D; MS) and analyzed by flow cytometry using the MACSQuant Analyzer.

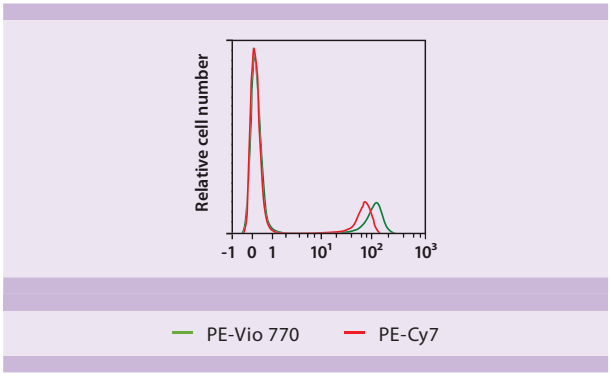

Figure 31: Analysis of human PBMCs using CD8 antibodies (clone: BW135/80) conjugated to either PE-Vio 770 or PE-Cy7. Concurrent staining with CD14-PerCP and CD56-PE was performed to exclude $CD14^+$ and $CD56^+$ cells from the analysis.

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Sample	Conjugate	MFI	Stain index	Compensation in channel B2 (%)
A	CD8-PE-Vio 770	109.2	97.3	0.4
A	CD8-PE-Cy7	67.0	72.7	2.2
B	CD8-PE-Vio 770	101.0	112.0	0.4
B	CD8-PE-Cy7	65.6	81.7	2.2

Table 15: MFI and stain indices of CD8-PE-Vio 770 and CD8-PE-Cy7.

Fixation stabilities

Tandem conjugates are usually less stable after fixation than single absorption/emission fluorochromes. However, PE-Vio 770 shows excellent stability as shown in figure 32. Cells fixed and stained with PE-Vio 770, in general, exhibit between a 0% and 7% loss in fluorescence, making them excellent candidates for fixation studies.

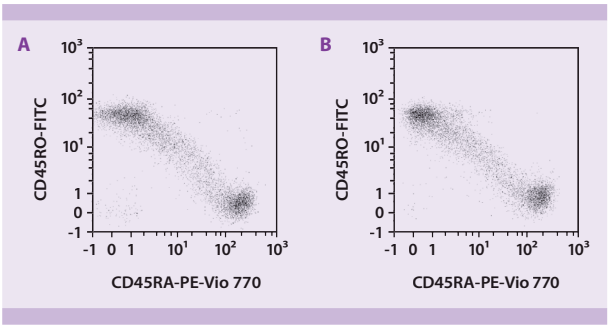
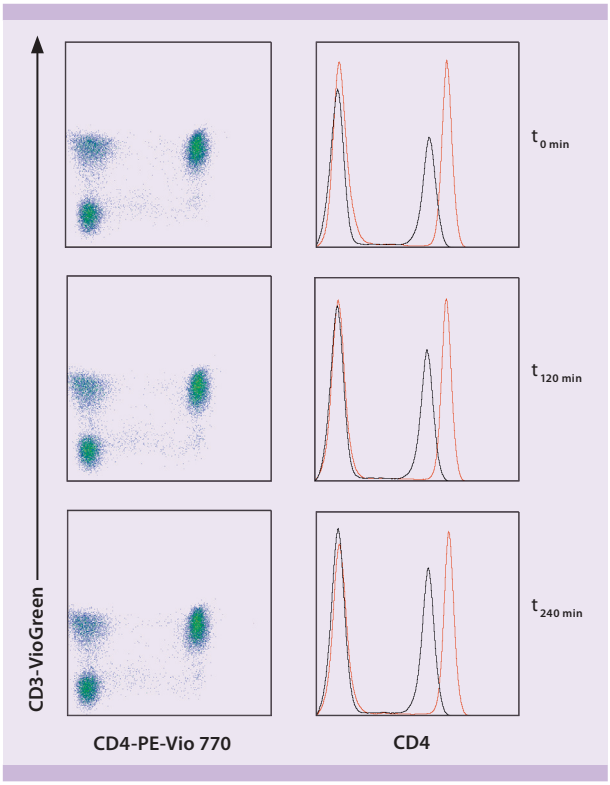


Figure 32: CD45RA-PE-Vio 770 fluorescence before (A) and after (B) paraformaldehyde fixation.

Photo-induced conjugate degradation

Analysis of the photo-induced degradation of CD4-PE-Vio 770 indicated no discernable changes after up to four hours of continuous exposure to ambient light (~850 Lux). Significantly higher mean fluorescence intensities (MFI) and stain indices (SI) for these time points, compare to commercially available alternatives (BD™ Biosciences), were also demonstrated (figure 33).



Marker	MFI			Stain index			Percent spillover (t_{0min} vs. t_{240min})		
	t_{0min}	t_{120min}	t_{240min}	t_{0min}	t_{120min}	t_{240min}	V1	B2	R1
CD4-PE-Vio 770	47	46	44	43	42	39	–	0.7/5.5	–
CD4-PE-Cy7	21	21	20	24	24	23	–	0.7/9.0	–

Figure 33: Photo-induced conjugate degradation of PE-Vio 770, with corresponding MFI and SI. Red lines indicate CD4-PE-Vio 770 (clone VIT-4.3) from Miltenyi Biotec. Black lines indicate CD4-PE-Cy7 (clone SK3) from BD Biosciences. Conjugates were exposed to ambient light for up to four hours, with negligible degradation rates.

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Red laser dyes

APC-Vio® 770 Tandem Conjugate

- Large Stokes shift dye, excited with the yellow (561 nm) or red (635 nm) laser, with an emission in the near-infrared region at 775 nm
- The combination of Vio® 770 as the acceptor dye and an optimized chemistry have furnished a tandem dye that is characterized by a high fluorescence intensity
- Minimal spillover to adjacent detection channels and low non-specific binding to non-target cells to meet the complexity of multiparameter cell analysis
- Higher MFI and stain index values, in addition to lower compensation settings, when compared to APC-Cy7

Fluorochrome	Absorption max. (nm)	Emission max. (nm)
APC-Vio 770	652	775
APC-Cy7	650	774
APC-Alexa Fluor 750	650	775

Table 16: Absorption and emission maximums of fluorochromes related to APC-Vio 770.

Laser and filter compatibility

The tandem dyes APC-Vio 770, APC-Cy7, and APC-H7 all use APC as the donor fluorochrome, showing maximum absorption at 652 nm. The emission spectra for Vio 770, Cy7, H7, and Alexa Fluor 750 are similar, making APC-Vio 770 an ideal tandem conjugate candidate for this channel in all flow cytometers (figure 34).

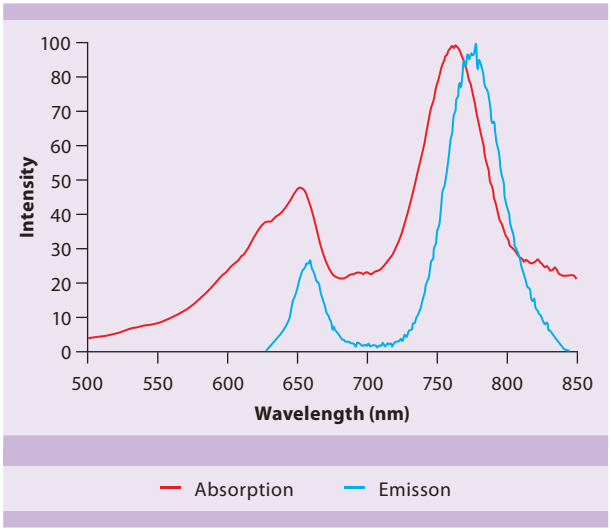

Figure 34: Absorption and emission spectra of APC-Vio 770.

Enhanced brightness

APC-Vio 770 provides strong fluorescent staining, allowing the identification and analysis of specific cellular populations (figure 35). When compared to other spectrally similar conjugates such as APC-Cy7 and APC-H7, APC-Vio 770 exhibits equal or stronger staining patterns (figure 36). In addition, APC-Vio 770 generally exhibits higher mean fluorescence intensities (MFI) and greater stain index values, and requires less compensation in the R1 channel than both APC-Cy7 and APC-H7 (table 15). These properties make APC-Vio 770 an ideal fluorochrome for use in the R2 channel.

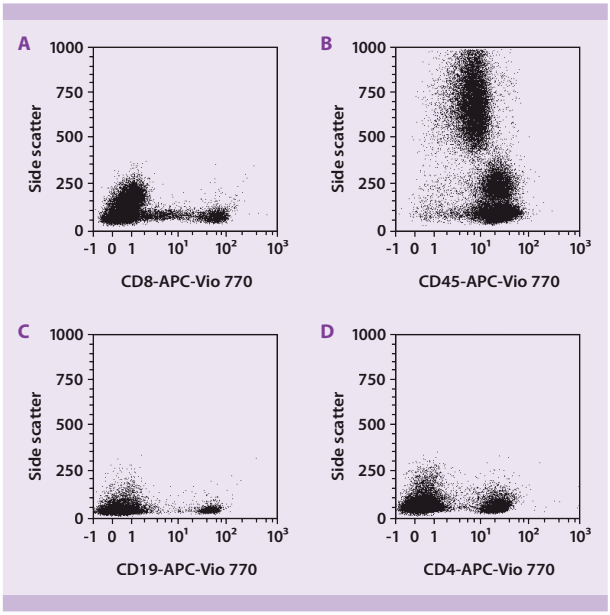

Figure 35: Human peripheral blood mononuclear cells (PBMC) or mouse splenocyte (MS) cells were stained with CD8-APC-Vio 770 (A; PBMCs), CD45-APC-Vio 770 (B; PBMCs), CD19-APC-Vio 770 (C; MS), or CD4-APC-Vio 770 (D; MS) and analyzed by flow cytometry using the MACSQuant Analyzer.

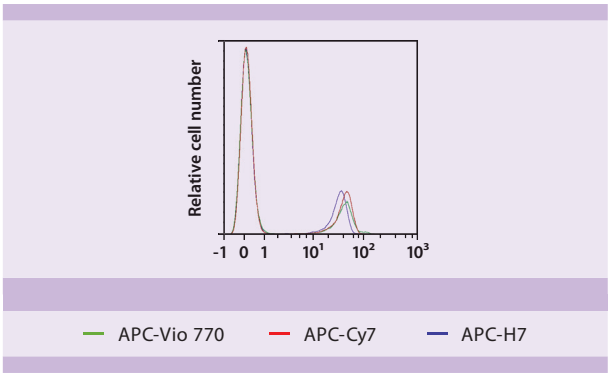

Figure 36: Analysis of human PBMCs using CD8 antibodies (clone BW135/80) conjugated to APC-Vio770, APC-Cy7, or APC-H7. Concurrent staining with CD14-PerCP and CD56-PE was performed to exclude $CD14^+$ and $CD56^+$ cells from the analysis.

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Sample	Conjugate	MFI	Stain index	Compensation in channel R1 (%)
A	CD8-APC-Vio 770	41.4	52.8	7.0
A	CD8-APC-Cy7	40.6	50.6	11.0
A	CD8-APC-H7	32.2	45.8	9.0
B	CD8-APC-Vio 770	39.4	57.8	7.0
B	CD8-APC-Cy7	38.8	58.5	11.0
B	CD8-APC-H7	31.2	51.8	9.0

Table 17: MFI and stain indices of CD8-APC-Vio 770, CD8-APC-Cy7, and CD8-APC-H7.

Fixation stabilities

APC-Vio® 770 shows excellent stability after fixation with paraformaldehyde (figure 37), similar to PE-Vio 770. Cellular material fixed with APC-Vio 770 exhibited, on average, a fluorescence decrease of between 5% and 10%, indicating APC-Vio 770's suitability and stability in fixation experiments.

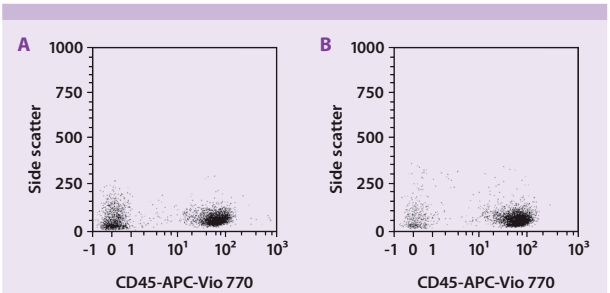
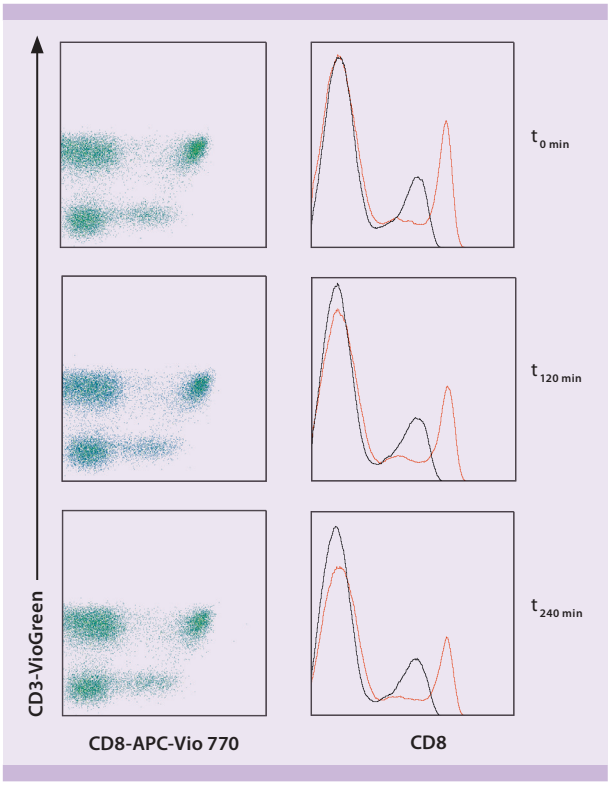


Figure 37: Cells were stained with CD45-APC-Vio 770 and left untreated (A) or fixed with paraformaldehyde (B).

Photo-induced conjugate degradation

Analysis of the photo-induced degradation of CD8-APC-Vio 770 indicated no discernable changes after up to 4 hours of continuous exposure to ambient light (~850 Lux). Significantly higher mean fluorescence intensities (MFI) and stain indices (SI) for these time points, compared to commercially available alternatives (BD Biosciences), were also demonstrated (figure 38).



Marker	MFI			Stain index			Percent spillover (t_{0min} vs. t_{240min})		
	t_{0min}	t_{120min}	t_{240min}	t_{0min}	t_{120min}	t_{240min}	V1	B2	R1
CD8-APC-Vio 770	41	41	39	24	23	22	–	–	9.0/15.0
CD8-APC-Cy7	15	15	15	11	11	11	–	–	9.0/16.0

Figure 38: Photo-induced conjugate degradation of APC-Vio 770, with corresponding MFI and SI. Red lines indicate CD8-APC-Vio 770 (clone BW 135/80) from Miltenyi Biotec. Black lines indicate CD8-APC-Cy7 (clone RPA-T8) from BD Biosciences. Conjugates were exposed to ambient light for up to four hours, with negligible degradation rates.

For a complete antibody product list refer to **www.miltenyibiotec.com/antibodies**

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Miltenyi Biotec

Germany/Austria/ Switzerland
Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone +49 2204 8306-0
Fax +49 2204 85197
macs@miltenyibiotec.de

USA/Canada
Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn, CA 95602, USA
Phone 800 FOR MACS
Phone +1 530 888 8871
Fax +1 877 591 1060
macs@miltenyibiotec.com

Australia
Miltenyi Biotec
Australia Pty. Ltd.
Unit 16A, 2 Eden Park Drive
Macquarie Park NSW 2113
Australia
Phone +61 2 8877 7400
Fax +61 2 9889 5044
macs@miltenyibiotec.com.au

Benelux
Miltenyi Biotec B.V.
Schipholweg 68 H
2316 XE Leiden
The Netherlands
macs@miltenyibiotec.nl
Customer service The Netherlands
Phone 0800 4020120
Fax 0800 4020100
Customer service Belgium
Phone 0800 94016
Fax 0800 99626
Customer service Luxembourg
Phone 800 24971
Fax 800 24984

China
Miltenyi Biotec Technology & Trading (Shanghai) Co., Ltd.
Room 2309
No. 319, Xianxia Road
Changning District
200051 Shanghai, P.R. China
Phone +86 21 62351005
Fax +86 21 62350953
macs@miltenyibiotec.com.cn

France
Miltenyi Biotec SAS
10 rue Mercoeur
75011 Paris, France
Phone +33 1 56 98 16 16
Fax +33 1 56 98 16 17
macs@miltenyibiotec.fr

Italy
Miltenyi Biotec S.r.l.
Via Persicetana, 2/D
40012 Calderara di Reno (BO)
Italy
Phone +39 051 6 460 411
Fax +39 051 6 460 499
macs@miltenyibiotec.it

Japan
Miltenyi Biotec K.K.
Nittsu-Eitai Building 5F
16-10 Fuyuki, Koto-ku,
Tokyo 135-0041, Japan
Phone +81 3 5646 8910
Fax +81 3 5646 8911
macs@miltenyibiotec.jp

Nordics and Baltics
Miltenyi Biotec Norden AB
Scheelevägen 17
223 70 Lund
Sweden
macs@miltenyibiotec.se
Customer service Sweden
Phone 0200-111 800
Fax 046-280 72 99
Customer service Denmark
Phone 80 20 30 10
Fax +46 46 280 72 99
Customer service Norway, Finland, Iceland, and Baltic countries
Phone +46 46 280 72 80
Fax +46 46 280 72 99

Singapore
Miltenyi Biotec Asia Pacific Pte Ltd.
100 Beach Road
#28-06 to 28-08 Shaw Tower
Singapore 189702
Phone +65 6238 8183
Fax +65 6238 0302
macs@miltenyibiotec.com.sg

South Korea
Miltenyi Biotec Korea Co., Ltd
Arigi Bldg. 8F
562 Nonhyeon-ro
Gangnam-gu
Seoul 06136, South Korea
Phone +82 2 555 1988
Fax +82 2 555 8890
macs@miltenyibiotec.co.kr

Spain
Miltenyi Biotec S.L.
C/Luis Buñuel 2
Ciudad de la Imagen
28223 Pozuelo de Alarcón (Madrid)
Spain
Phone +34 91 512 12 90
Fax +34 91 512 12 91
macs@miltenyibiotec.es

United Kingdom
Miltenyi Biotec Ltd.
Almac House, Church Lane
Bisley, Surrey GU24 9DR, UK
Phone +44 1483 799 800
Fax +44 1483 799 811
macs@miltenyibiotec.co.uk

www.miltenyibiotec.com

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