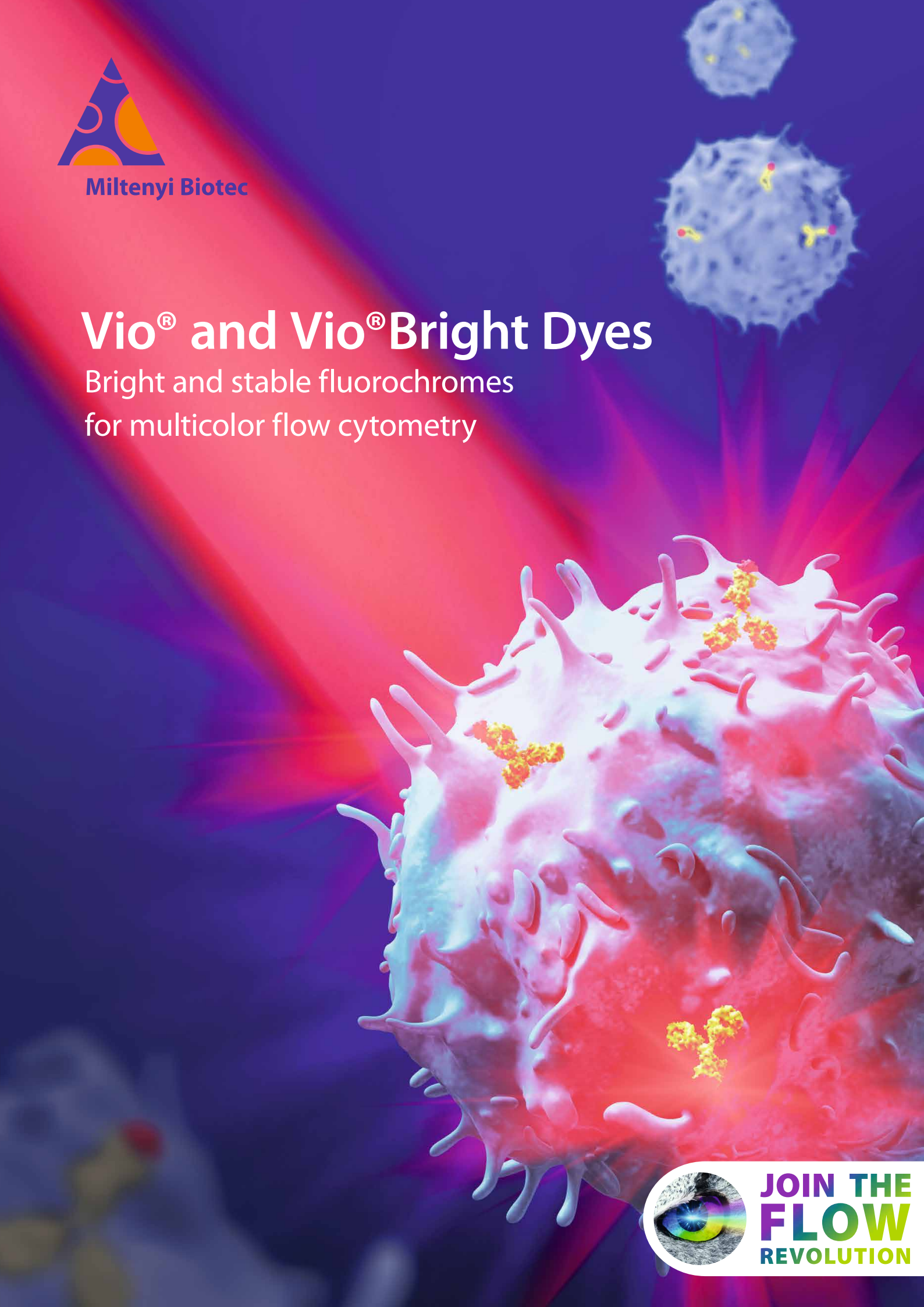
Miltenyi Biotec

Vio® and Vio®Bright Dyes

Bright and stable fluorochromes for multicolor flow cytometry



JOIN THE FLOW REVOLUTION

Bright and stable fluorochromes for multicolor flow cytometry

Vio® and Vio®Bright Dyes represent a family of fluorochromes for flow cytometry and fluorescence microscopy. They are characterized by high fluorescence intensities and low spillover, making them an ideal choice for multicolor flow applications.

Combined with traditional fluorochromes, such as FITC, PE, PerCP, and APC, Vio and VioBright Dyes expand available dye options, giving you more flexibility in setting up multiparameter cell analyses.

Fluorochrome-conjugated MACS® Antibodies are perfectly suited for identification and enumeration of human, mouse, rat, or non-human primate cells.

For more information, visit
www.miltenyibiotec.com/vio

REAfinity™ Recombinant Antibodies – Flow cytometry is in their genes

When used in combination with our proprietary REAfinity Recombinant Antibodies, you can take advantage of superior lot-to-lot consistency and purity compared to mouse or rat monoclonal and polyclonal antibodies.

Our recombinant technology also diminishes the need for FcR blocking and allows for analyses with only one single isotype control, generating high quality data with no more background signal while saving efforts when setting up experiments.

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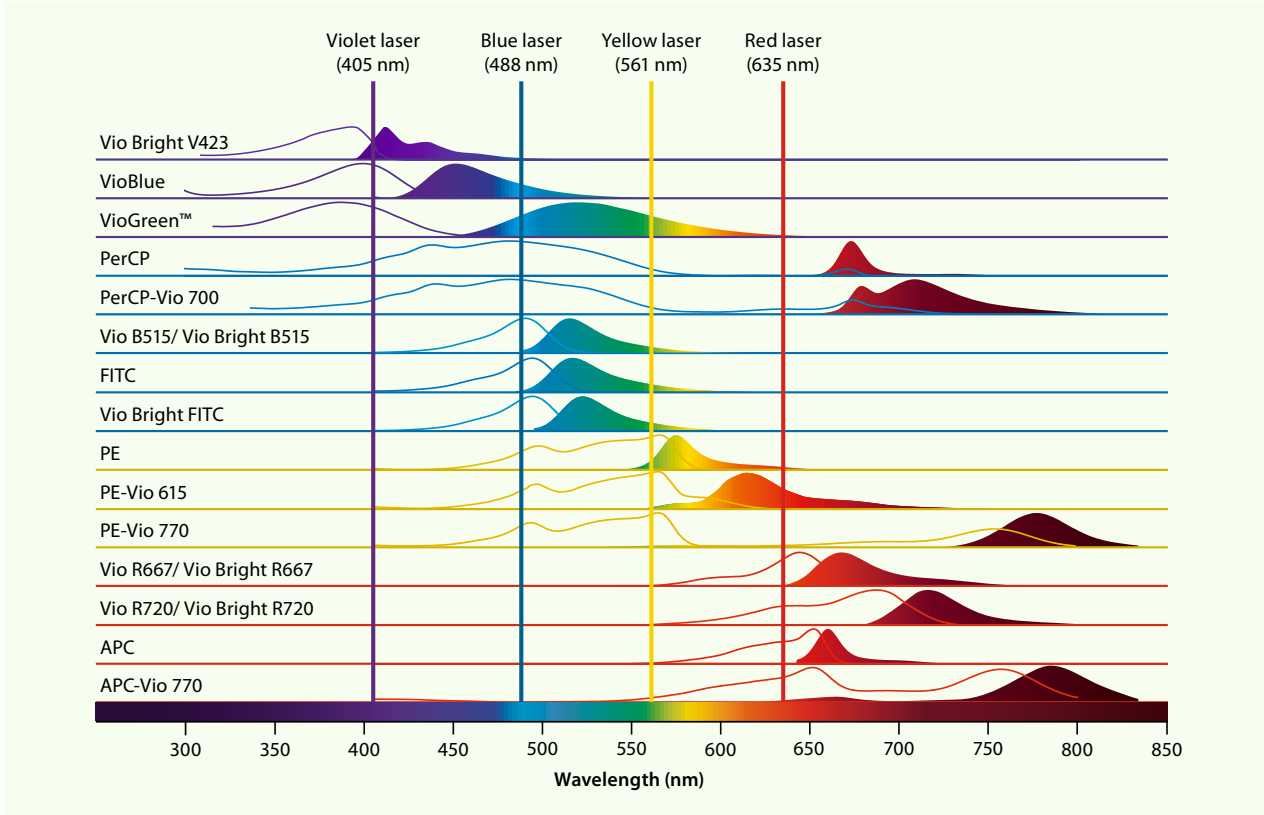
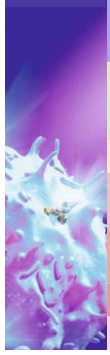
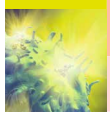


Fluorochrome guide

	Common filters (nm)	Miltenyi Biotec dyes	Brightness (MFI)	Other dye examples		Viability dyes
Violet 405 nm 	450/50	**VioBlue®** **Vio® Bright V423**	3 5	Alexa Fluor® 405 BD Horizon™ V450 BD Horizon Brilliant Violet™ (BV) 421 Calcein Violet 450 AM Cascade Blue®	Vybrant® DyeCycle™ Violet eBFP eFluor® 450 Pacific Blue™ Super Bright 436	Viobility™ 405/452 Fixable Dye DAPI Hoechst 33342 Zombie Violet™
	525/50	**VioGreen™**	1	Alexa Fluor® 430 BD Horizon™ V500 BD Horizon™ BV510	Cascade Yellow™ Krome Orange™	Viobility 405/520 Fixable Dye AmCyan Zombie Aqua™
	579/34			BD Horizon™ BV570	Pacific Orange™	
	605/15 or 615/20			BD Horizon™ BV605	Super Bright 600	
	661/11 or 667/30			BD Horizon™ BV650	Super Bright 645	
	711/25			BD Horizon™ BV711	Super Bright 702	
	785/60			BD Horizon™ BV786		
Blue 488 nm 	525/50	**FITC** **Vio® B515** **Vio® Bright** **Vio® Bright B515**	3 4 4 5	Alexa Fluor® 488 Calcein AM DyLight® 488 BD Horizon Brilliant™ Blue (BB) 515	CFSE GFP, YFP SYTOX® Green Vybrant® DyeCycle™ Green	Viobility 488/520 Fixable Dye Zombie Green™
	585/40 or 579/34	**PE**	5	Cy™ 3	Vybrant® DyeCycle™ Orange	CyTRAK Orange™
	655–730	**PerCP** **PerCP-Vio® 700**	1 3	PE-Cy™ 5 PE-Cy™ 5.5 PerCP-Cy™ 5.5	PerCP-eFluor® 710 BD Horizon™ BB700	Propidium iodide (PI) 7-AAD CyTRAK Orange™
	655–730 or 615/20	**PE-Vio 615**	5	ECD PE/Texas Red® PE-CF594	PE/Dazzle™ 594 PE-eFluor® 610	
	750 LP or 785/62	**PE-Vio 770**	4	PE-Alexa Fluor® 750	PE-Cy™ 7	
Yellow-green 561 nm 	586/15	**PE**	5	Cy™ 3	Vybrant® DyeCycle™ Orange	PI CyTRAK Orange™
	615/20	**PE-Vio 615**	5	ECD PE/Texas Red® PE-CF594	PE/Dazzle™ 594 PE-eFluor® 610	
	750 LP	**PE-Vio 770**	4	PE-Alexa Fluor® 750	PE-Cy™ 7 RFP	DRAQ7®
Red 635 nm 	655–730	**APC** **Vio® R667** **Vio® Bright R667**	4 5 5	Alexa Fluor® 647 Cy™ 5	eFluor® 660	DRAQ5® DRAQ7®
	720/40	**Vio R720** **Vio Bright R720**	3 4	Alexa Fluor® 700 APC-Alexa Fluor® 700	APC-R700	DRAQ5® DRAQ7®
	750 LP or 785/62	**APC-Vio 770**	3	APC-Alexa Fluor® 750 APC-Fire™ 750 APC-Cy™ 7	APC-eFluor® 780 APC-H7	DRAQ5® DRAQ7® Zombie NIR™

Violet laser fluorochromes

Vio® Bright V423, VioBlue® and VioGreen™ dyes are fluorochromes that can be excited by the violet laser. VioBlue and VioGreen are small molecule dyes that show advances in brightness and signal-to-noise ratios (fig. 1), while reducing intralaser compensation requirements. Both fluorochromes are exceptionally stable during fixation and show high photo stability, making them an ideal choice for fluorescent microscopy.

Vio Bright V423: Our brightest option for violet laser

Vio Bright V423 is a synthetic dye which is 5× brighter than VioBlue dye. Compared to polymer-based fluorochromes, Vio Bright V423 exhibits a similar or brighter degree of fluorescence (fig. 2). Additionally, minimal spillover and excellent resolution of rare cells and dim antigens make it a superior choice for multiparameter cell analysis.

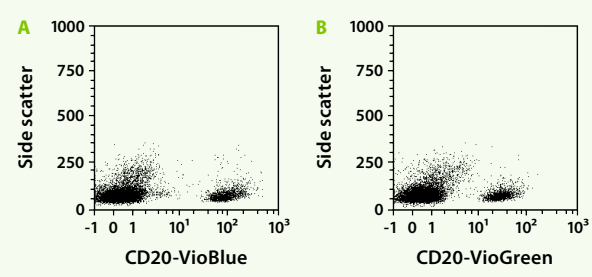


Figure 1: Human PBMCs were stained with CD20-VioBlue (A), CD20-VioGreen (B), or CD8-VioBlue (C). Mouse splenocytes were stained with CD8a-VioGreen (D). Samples were analyzed by flow cytometry using the MACSQuant® Analyzer 10.

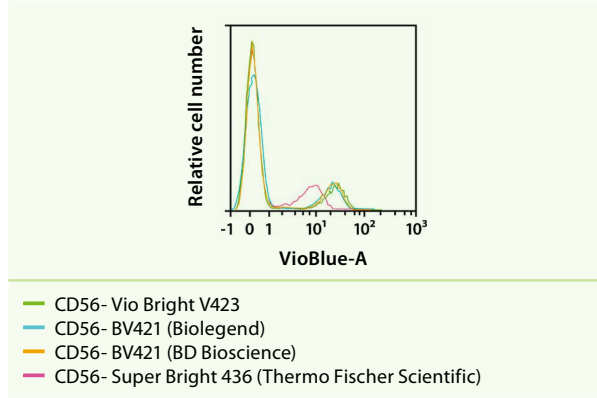


Figure 2: Fluorescence intensity of human PBMCs stained with CD56 antibody conjugated to Vio Bright V423, Brilliant Violet 421 (Biolegend), Brilliant Violet 421 (BD Bioscience) and Super Bright 436 (Thermo Fischer Scientific).

	Brightness	Fixation stability	Microscopy	Photostability	Excitation max	Emission max
Vio Bright V423	■■■■■	PFA, methanol	Not tested	High	399 nm	420 nm
VioBlue	■■■□□	PFA, ethanol, methanol, and acetone	Not tested	Medium	400 nm	452 nm
VioGreen	■□□□□	PFA, ethanol, methanol, and acetone	Not tested	High	388 nm	520 nm

Blue laser fluorochromes

Vio® Bright FITC and Vio Bright B515 are revolutionary blue laser dyes, developed exclusively by Miltenyi Biotec. The innovative Vio Bright 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.

Brighter alternatives to FITC

Vio Bright FITC and Vio Bright B515 are bright alternatives for the FITC channel. Their excellent brightness makes them a good choice for the detection of low-expressed markers, rare cells, as well as dim or uncharacterized markers.

Dye	Features	Application
Vio Bright FITC (1st generation Vio Bright dye)	• High brightness	Detection of extracellular markers
Vio Bright B515 (2nd generation Vio Bright dye)	• Very high brightness • Low spillover • High fixation & photostability	Detection of extracellular markers
Vio® B515 (Organic dye)	• Moderate brightness, • Low spillover • High fixation & photostability	Detection of intracellular markers

Table 1: Features and applications for Vio Bright FITC, Vio Bright B515, and Vio B515 in comparison.

High photostability, no matter the fixation reagent

Bright fluorochrome conjugates, such as PE and APC, are often sensitive to fixation reagents. For a successful flow cytomeric analysis, which often involves staining of intracellular markers, stability of fluorochrome conjugates is critical.
Vio Bright B515 shows excellent stability to methanol- and paraformaldehyde-based fixatives with minimal compromises in brightness (fig. 3).

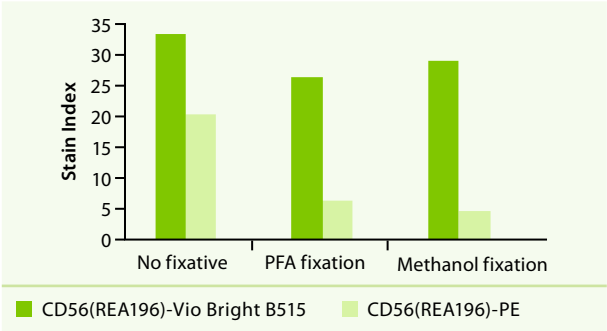


Figure 3: Human PBMCs were stained using CD56 antibodies (clone REA 196) conjugated to Vio Bright B515 or PE. In addition, cells were analyzed before and after fixation with 3.7% paraformaldehyde and 90% methanol. Stained cells were analyzed by flow cytometry using the MACSQuant® Analyzer 10.

	Brightness	Fixation stability	Microscopy	Photostability	Excitation max	Emission max
Vio B515	■■■■□	PFA, ethanol, methanol, and acetone	Recommended	Very high	488 nm	514 nm
Vio Bright FITC	■■■■□	PFA, ethanol, methanol, and acetone	Suitable	Low	496 nm	522 nm
Vio Bright B515	■■■■■	PFA, ethanol, methanol, and acetone	Suitable	Medium	488 nm	514 nm

Blue and yellow-green laser tandem conjugates

All blue laser tandem conjugates provide excellent brightness for confident detection of dim, rare, and uncharacterized markers. PE-Vio® 615, PE-Vio 770, and PerCP-Vio 700 are tandem conjugates with PE or PerCP as donor dyes, while Vio 615/770/700 act as acceptor dyes. These tandem fluorochromes are optimized for efficient donor-to-acceptor dye energy transfer, high fluorescent intensity, and low spillover into the donor dye detection channel.

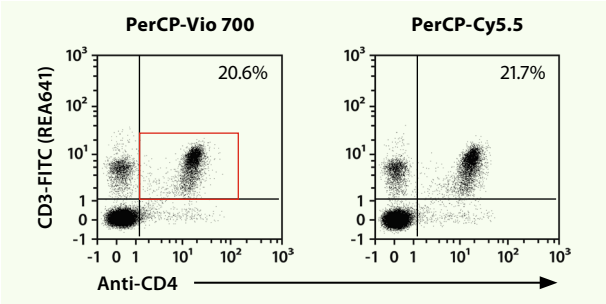


Figure 4: Mouse splenocytes were stained with CD4 antibodies (clone GK.1) conjugated to either PerCP-Vio 700 or PerCP-Cy5.5. Concurrent staining with CD3-FITC (REA641) was performed to identify CD4⁺ T cells.

PE-Vio 770 – the brightest of them all

PE-Vio 770 provides the greatest fluorescence intensity of the Vio Dye family, with excellent separation of positively and negatively stained cells. When compared to other spectrally similar tandem conjugates, such as PE-Cy7, PE-Vio 770 exhibits significantly higher fluorescence intensities (fig. 5).

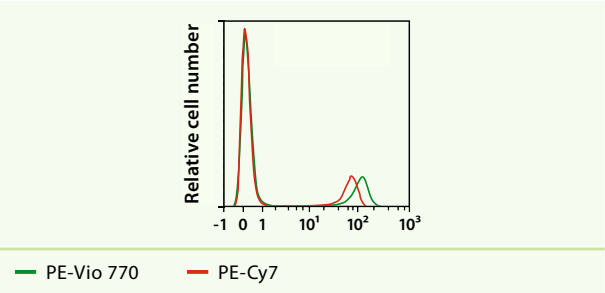


Figure 5: Analysis of human PBMCs using CD8 antibodies (clone BW135/80) conjugated to either PE-Vio 770 or PE-Cy7. Concurrent staining with CD14-PerCP and CD56-PE was performed to exclude CD14⁺ and CD56⁺ cells from the analysis.

	Brightness	Fixation stability	Microscopy	Photostability	Excitation max	Emission max
PerCP-Vio 700	■■■□□	PFA	Not tested	Not tested	482 nm	704 nm
PE-Vio 615	■■■■■	PFA	Not tested	Not tested	565 nm	620 nm
PE-Vio 770	■■■■□	PFA	Not tested	Not tested	565 nm	775 nm

Red laser fluorochromes

The APC-Vio® 770 Dye is a tandem conjugate, like APC-Cy7 or APC-H7, consisting of fluorescence donor (APC) and acceptor (Vio 770).

APC-Vio 770 provides strong fluorescent staining for an accurate identification and analysis of specific cellular populations. Compared to other spectrally similar conjugates APC-Vio 770 exhibits equal or stronger staining patterns (fig. 6).

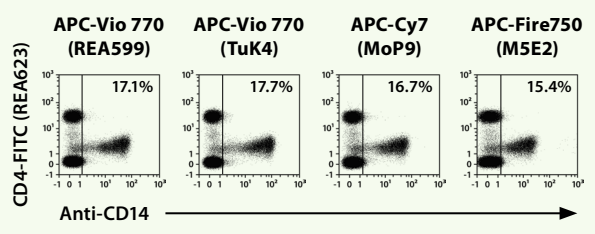


Figure 6: Human PBMCs were stained with CD14 antibodies conjugated to either APC-Vio 770, APC-Cy7, or APC-Fire750. Concurrent staining with CD4-FITC (REA623) was performed to identify $CD4^+CD14^+$ monocytes.

Vio® Bright R667 is the brightest fluorochrome for the red laser, allowing for improved separation of negative and positive cell populations - great for detection of dim markers.
Vio Bright R667 is a superior alternative to the APC conjugate with a 2-fold increase in brightness over APC. Its high fixation and photo stability make it an excellent choice for microscopy applications (fig. 7).

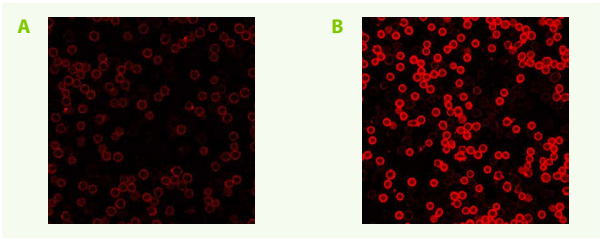


Figure 7: Confocal microscopy analysis of PBMCs stained with CD4 antibodies conjugated to either Alexa Fluor 647 (A) or Vio Bright R667 (B).

Brighter alternative to Alexa-700

VioBright multimerization technology results in 2-fold or higher increase in brightness of Vio Bright R720 compared to conventional fluorophore like Alexa Fluor 700 (fig. 8). This dye also shows significant reduction in background noise.

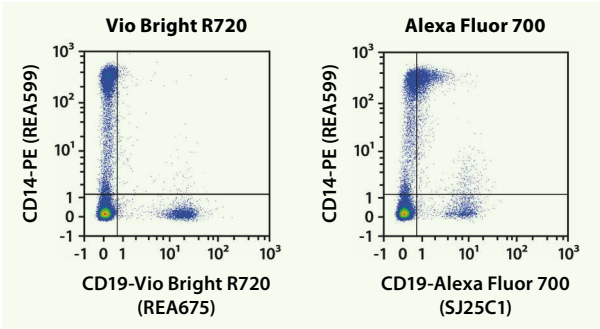


Figure 8: Human PBMCs were stained with CD19 conjugated with Vio Bright R720 or Alexa Fluor 700 and analyzed by the MACSQuant® 16.

	Brightness	Fixation stability	Microscopy	Photostability	Excitation max	Emission max
APC-Vio 770	■■■□□	PFA	Not tested	Not tested	652 nm	775 nm
Vio R667	■■■■■	PFA, ethanol, methanol, and acetone	Recommended	Very high	645 nm	668 nm
Vio Bright R667	■■■■■+	PFA, ethanol, methanol, and acetone	Suitable	Medium	645 nm	668 nm
Vio R720	■■■□□	PFA, ethanol, methanol	Not tested	High	695 nm	720 nm
Vio Bright R720	■■■■□	PFA, ethanol, methanol	Not tested	High	695 nm	720 nm

▸ miltenyibiotec.com/vio

Miltenyi Biotec

Germany/Austria
Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone +49 2204 8306-0
Fax +49 2204 85197
macsde@miltenyi.com

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
macsus@miltenyi.com

Australia
Miltenyi Biotec
Australia Pty. Ltd.
Unit 11, 2 Eden Park Drive
Macquarie Park, NSW 2113
Australia
Phone +61 2 8877 7400
Fax +61 2 9889 5044
macsau@miltenyi.com

Benelux
Miltenyi Biotec B.V.
Sandifortdreef 17
2333 ZZ Leiden
The Netherlands
macsnl@miltenyi.com
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 401
No. 1077, Zhangheng Road
Pudong New Area
201203 Shanghai, P.R. China
Phone +86 21 6235 1005
Fax +86 21 6235 0953
macscn@miltenyi.com

France
Miltenyi Biotec SAS
10 rue Mercoeur
75011 Paris, France
Phone +33 1 56 98 16 16
Fax +33 1 56 98 16 17
macsfr@miltenyi.com

Italy
Miltenyi Biotec S.r.l.
Via Paolo Nanni Costa, 30
40133 Bologna
Italy
Phone +39 051 6 460 411
Fax +39 051 6 460 499
macsit@miltenyi.com

Japan
Miltenyi Biotec K.K.
NEX-Eitai Building 5F
16-10 Fuyuki, Koto-ku
Tokyo 135-0041, Japan
Phone +81 3 5646 8910
Fax +81 3 5646 8911
macsjp@miltenyi.com

Nordics and Baltics
Miltenyi Biotec Norden AB
Medicon Village
Scheeletorget 1
223 81 Lund
Sweden
macsse@miltenyi.com
Customer service Sweden
Phone 0200 111 800
Customer service Denmark
Phone 80 20 30 10
Customer service
Norway, Finland, Iceland,
and Baltic countries
Phone +46 46 280 72 80

Singapore
Miltenyi Biotec Asia Pacific Pte Ltd
438B Alexandra Road, Block B
Alexandra Technopark
#06-01
Singapore 119968
Phone +65 6238 8183
Fax +65 6238 0302
macssg@miltenyi.com

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
macskr@miltenyi.com

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
macses@miltenyi.com

Switzerland
Miltenyi Biotec Swiss AG
Gibelinstrasse 27
4500 Solothurn
Switzerland
Phone +41 32 623 08 47
Fax +49 2204 85197
macsch@miltenyi.com

United Kingdom
Miltenyi Biotec Ltd.
Almac House, Church Lane
Bisley, Surrey GU24 9DR, UK
Phone +44 1483 799 800
Fax +44 1483 799 811
macsuk@miltenyi.com

www.miltenyibiotec.com

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

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