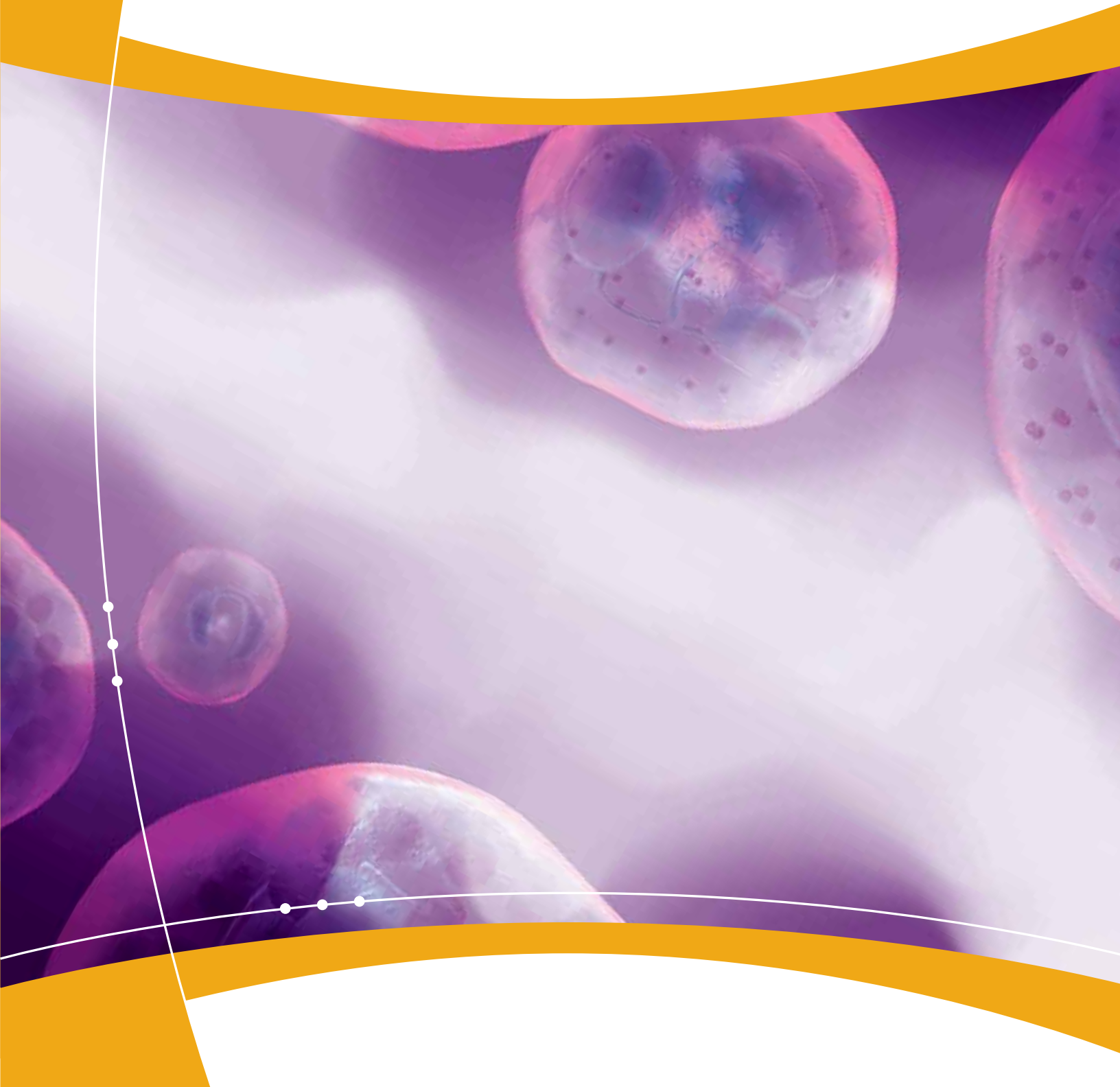
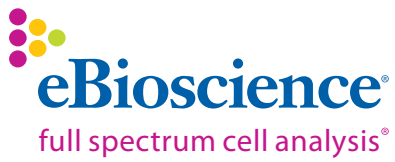




Violet Laser Reagents

The eFluor[®] Solution





eBioscience is committed to developing and manufacturing high-quality, innovative reagents in an ISO certified facility. As a provider of more than 10,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in the areas of innovation, quality and value.



VIOLET LASER REAGENTS: The eFluor™ Solution

Introduction

Flow cytometry is increasingly recognized as a valuable tool for deciphering complex cellular processes and interactions in a variety of systems that model normal and disease states. The evolution of flow cytometry as a fundamental tool for the life scientist has been fueled by technological advances in instrumentation that include the introduction of a violet laser as a standard component of multi-laser flow cytometers. These advances in instrumentation have underscored the need for high quality reagents that allow realization of the power of multicolor flow cytometry.

To better support violet laser-based multicolor cytometry, eBioscience has developed a line of eFluor™ reagents compatible with the violet laser (405nm) line. The eFluor™ brand includes both nanocrystal and organic dye-derived fluorescent molecules that have been paired with appropriate antibody specificities to maximize the amount of information that can be acquired from a valuable sample (Figure 1). We currently offer over 100 reagents optimized for use with the violet laser.

The growing eFluor™ brand now includes the following fluorophores for use with the violet laser:

eFluor™ 450: an organic dye that is an alternative to Pacific Blue® in the eBioscience portfolio. eFluor™ 450 emission is slightly red shifted and has a narrower profile compared to Pacific Blue® resulting in less overall compensation.

eFluor™ 605 Nanocrystals: a brighter alternative to Pacific Orange® that features very narrow emission spectra to maximize signal to noise and minimize compensation.

eFluor™ 650 Nanocrystals: our newest and brightest nanocrystal represents a third fluor for the violet laser. In contrast to AmCyan, eFluor™ 650^{NC} features a very narrow emission profile that maximizes signal to noise and minimizes compensation requirements on the violet laser.



eFluor™ Reagents for the Violet Laser

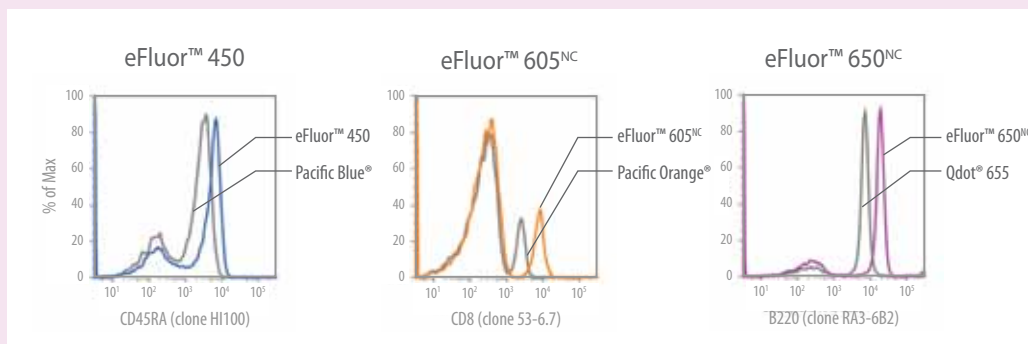


Figure 1: eFluor™ reagents for the violet laser. Staining for each fluorophore illustrates quality performance.

LEFT: Human peripheral blood cells were stained with eFluor™ 450 anti-human CD45RA or the same antibody clone conjugated to Pacific Blue®.

MIDDLE: Mouse splenocytes were stained with eFluor™ 605^{NC} anti-mouse CD8 or the same clone conjugated to Pacific Orange®. Fluorescent emission was collected through the same detector. Filters were changed for optimal detection of each fluorochrome as follows: 557 LP and 575/26 BP (Pacific Orange), 595 LP and 605/40 BP (eFluor™ 605^{NC}).

RIGHT: Mouse splenocytes were stained with eFluor™ 650^{NC} anti-mouse B220 or the same clone conjugated to Qdot® 655.

The eFluor™ Solution for the Violet Laser

The eFluor™ solution for the violet laser provides three fluorophores that work together to provide maximum resolution of antigenic determinants when used simultaneously. This is accomplished by narrow emission peaks with little spectral overlap and is in stark contrast to the broad emission peaks and spectral overlap seen with the traditional approach for the violet laser (Figure 2). Our broad portfolio of biological content permits ideal pairing of specificity with fluorophore that allows straightforward solutions for the violet laser.

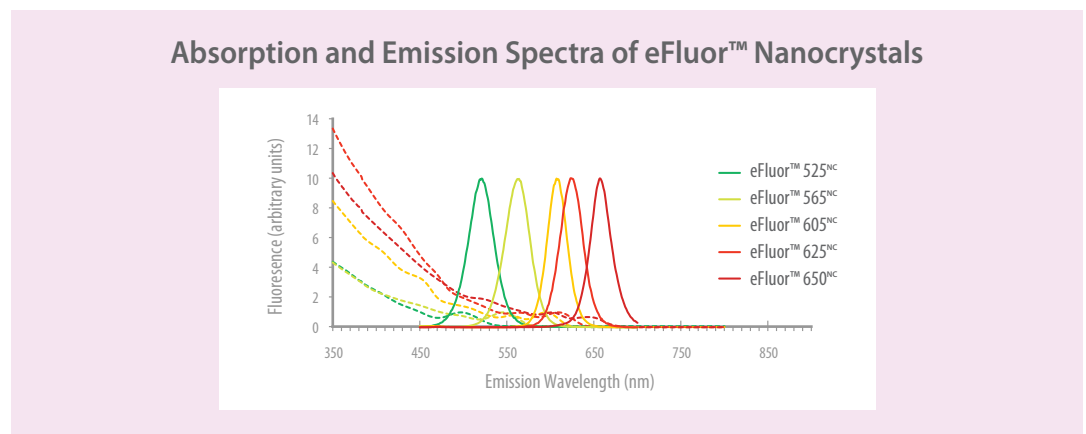
Figure 2: Emission spectra of traditional and eFluor™ reagents for the violet laser. Normalized emission spectra of the traditional fluorophores commonly used with the violet laser (top) versus the eFluor™ solution (bottom). The advantageous narrow emission spectra of the eFluor™ solution is evident when compared to the broad emission spectra and large spectral overlap of the traditional approach. The recommended collection filters for each fluorophore are indicated.



The eFluor™ Nanocrystals Advantage

The narrow emission profiles of the eFluor™ solution are derived from the properties of eFluor™ Nanocrystals. The semiconductor material-based particles are approximately protein sized (2-10nm) and behave as a fluorophore in biological applications. Their unique spectral properties include preferential excitation at shorter wavelengths (Figure 3) and a tendency towards extremely long stoke shifts.

Figure 3: Absorption and emission spectra. The absorption (dashed line) and emission (solid line) spectra of the range of eFluor™ Nanocrystals.



eBioscience eFluor™ Nanocrystals are constructed in a three step process (Figure 4). First, the wavelength specific core particle is synthesized and then sealed and protected with a Zinc-Sulfide shell (Step A). The second step involves the encapsulation of nanocrystals in a proprietary lipid coating (Step B) that ensures low background staining and also provides a stable platform for the final step of covalent coupling of antibodies to create each unique reagent (Step C).

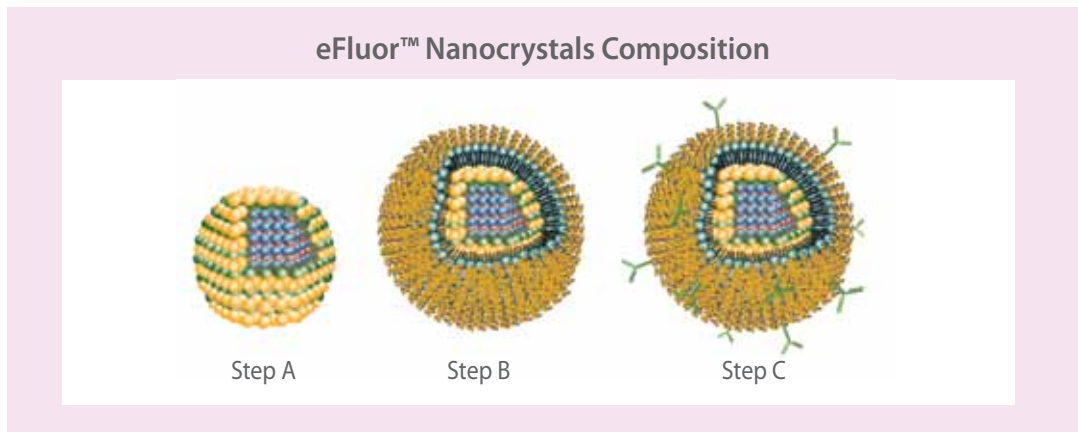


Figure 4: eFluor™ Nanocrystals composition. An illustration of the layered composition of eFluor™ Nanocrystals particles designed for life science applications.

Only eFluor™ Nanocrystals feature a unique proprietary coating that confers a combination of low fluorescence background and substantial elimination of aggregate phenomena often associated with other nanocrystal preparations.

Designing an Effective Multicolor Solution

An effective multicolor reagent panel is one that is robust in use and provides effective resolution for reproducible and accurate biological assessment. The use of the so called “Stain Index”, or SI, is an objective measure of how well resolved a given cell population is when analyzed using a particular instrument with its unique configuration and settings. Figure 5 graphically illustrates the SI calculation (left) and the actual SI values for eBioscience anti-mouse CD4 reagents (right). The SI is calculated as the difference in the means of the positive and negative populations (D) divided by two times the standard deviation of the negative peak (W). As can be seen in Figure 5, the applied compensation will increase the fluorescence spread of the negative population and subsequently decrease the resolution of the positive population from the negative.

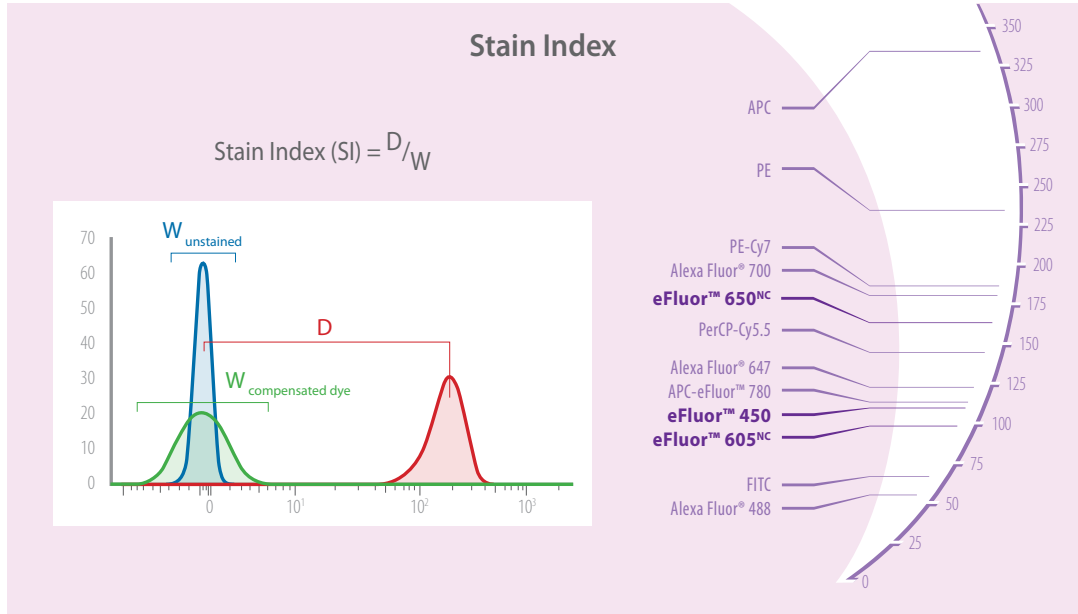


Figure 5: Stain Index - a measure of staining resolution. A graphical representation of the stain index calculation is shown on the left. The graphic on the right represents the calculated stain index for anti-mouse CD4 conjugated to the range of eBioscience fluorophores and used to stain mouse splenocytes.

eFluor™ Provides a Better Relative Stain Index

A simple comparison experiment was performed to assess the efficacy of the eFluor™ solution vs. the traditional approach. Human PBMC were stained for CD3, CD4, and CD8 using antibodies directly conjugated to traditional fluorophores (traditional approach) or eFluor™ fluorophores (eFluor™ solution). Samples were stained according to manufacturers recommended conditions, and acquired on a violet laser (50mW) equipped flow cytometer (BD LSRII), with compensation and filter sets optimized for each set of fluorophores. The resulting data plots and compensation values are shown in **Figure 6**.

Using the traditional approach, considerable amounts of spectral compensation were required in order to acquire usable data. Data from cells stained using the traditional approach is shown below (**Figure 6A**). A consequence of the compensation requirement is that significant data “spread” in the CD4 population and a decrease in CD8 resolution are observed that result from spillover of the AmCyan signal into the detectors for Pacific Blue® and Pacific Orange® respectively. The cumulative effect is a greatly reduced overall stain index (**Figure 7**). In contrast, cells stained using the eFluor™ solution (**Figure 6B**) require minimal compensation and the data clearly shows brighter CD8 staining with resolution of CD8 dim/NK cells, an absence of data spread, and the ability to unambiguously identify the CD4/CD8 double positive T cells that are not resolved when traditional approaches are employed.

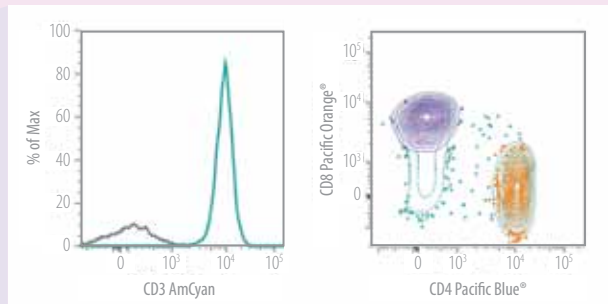
Figure 6: Comparison of the “traditional approach” with the eFluor™ solution. Human PBMC were stained with the traditional fluorophores AmCyan anti-human CD3, Pacific Blue® anti-human CD4, and Pacific Orange® anti-human CD8 (A) or with the eFluor™ solution of eFluor™ 650^{NC} anti-human CD3, eFluor™ 450 anti-human CD4, and eFluor™ 605^{NC} anti-human CD8 (B). The required compensation values are shown in table form to the left of the data plots.

Stain	Minus % Fluorochrome in Detector		
	Pacific Blue®	AmCyan	Pacific Orange®
CD4 Pacific Blue®		13.41	3.80
CD3 AmCyan	23.62		34.37
CD8 Pacific Orange®	1.32	77.84	

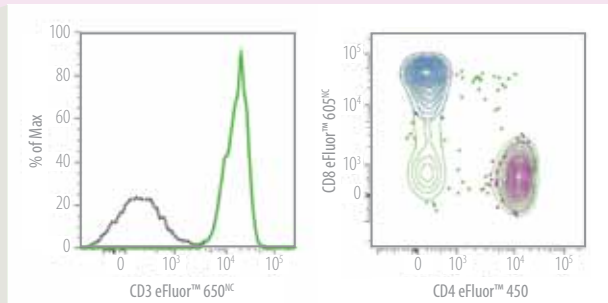
Stain	Minus % Fluorochrome in Detector		
	eFluor™ 450	eFluor™ 605 ^{NC}	eFluor™ 650 ^{NC}
CD4 eFluor™ 450		2.24	0.18
CD8 eFluor™ 605 ^{NC}	0.00		1.83
CD3 eFluor™ 650 ^{NC}	0.00	3.45	

Comparison of the “Traditional Approach” with the eFluor™ Solution

A: Traditional Approach



B: eFluor™ Solution



In order to objectively measure the visually superior performance of the eFluor™ solution in this multicolor example, stain indexes were calculated to quantitatively assess the benefits of the eFluor™ approach (Figure 7). The eFluor™ solution offered improved resolution for each of the three specificities evaluated for detection with the violet laser. The most significant advantage in this panel was the improved resolution of the CD8 population. The ongoing expansion of the eFluor™ product line from eBioscience brings researchers the needed tools to capitalize on their instrument investment by providing reagents to take advantage of the full capacity of their cytometer.

Relative Stain Index			
Specificity	Fluor	Relative Stain Index	% eFluor™ Advantage
CD3	eFluor™ 650 ^{NC}	34	13 %
	AmCyan	30	
CD4	eFluor™ 450	79	52 %
	Pacific Blue®	52	
CD8	eFluor™ 605 ^{NC}	51	219 %
	Pacific Orange®	16	

Figure 7: Relative stain index of the eFluor™ solution versus traditional fluorophores. From the data shown in Figure 6, the relative stain index was calculated from the compensated data for each antibody used in the staining panels. The percent improvement offered by the eFluor™ reagents over the traditional fluorophores is shown for each specificity.

CONCLUSION: eFluor™ Solution - Tailored for the Violet Laser

The development of flow cytometers equipped with a violet laser has helped drive recognition of multi-parameter flow cytometry as a “must have” tool for the life sciences. Realizing the potential of the violet laser requires robust and reliable reagents as well as relevant specificities for easy integration into any multicolor staining panel. A primary goal of eBioscience is to combine our industry leading expertise with our innovative spirit to develop optimized reagents for multicolor flow cytometry.

In order to validate performance of the eFluor™ solution for the violet laser, a three color staining panel consisting of CD3 eFluor™ 450, CD45RA eFluor™ 605^{NC}, and CD45RO eFluor™ 650^{NC} was used to discriminate human naïve and memory T cells. As shown in Figure 8, this panel easily resolved the CD3-expressing cells and showed the expected distribution of CD45RA and CD45RO.

The eFluor™ solution is an optimized fluorophore solution designed to maximize the power of your violet laser-equipped cytometer. By combining new organic dyes, proprietary biocompatible nanocrystal technologies and an extensive antibody portfolio, eBioscience has developed a uniquely powerful fluorescent reagent system for the violet laser.

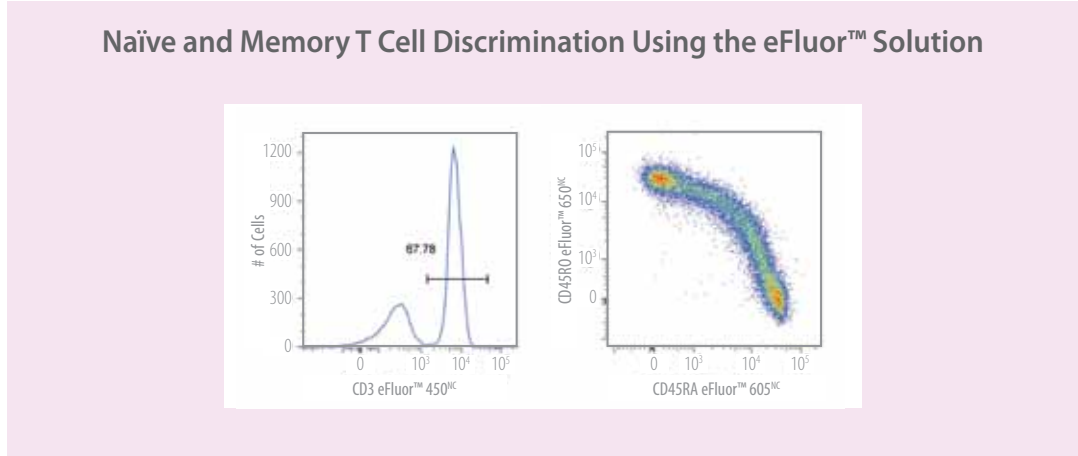


Figure 8: Naïve and memory T cell discrimination using the eFluor™ solution. Human PBMC were stained with eFluor™ 450 anti-human CD3, eFluor™ 605^{NC} anti-human CD45RA, and eFluor™ 650^{NC} anti-human CD45RO to visualize naïve and memory CD3+ T cells.

NOW AVAILABLE: Products for the Violet Laser

All Products are for research use only.

Human						
Description	Clone	Isotype	eFluor™ 450	Pacific Blue®	eFluor™ 605 ^{NC}	eFluor™ 650 ^{NC}
CD1a	HI149	mlgG1		57-0019		
CD3	OKT3	mlgG2a	48-0037	57-0037	93-0037	95-0037
	UCHT1	mlgG1	48-0038			
CD4	OKT4	mlgG2b		57-0048	93-0048	95-0048
	RPA-T4	mlgG1	48-0049			
CD8a	OKT8	mlgG2a		57-0086		
CD8a	RPA-T8	mlgG1	48-0088		93-0088	95-0088
CD11b	ICRF44	mlgG1			93-0118	
CD14	61D3	mlgG1	48-0149		93-0149	
CD15	HI98	mlgM		57-0159		
CD16	CB16	mlgG1	48-0168		93-0168	
CD19	HIB19	mlgG1	48-0199	57-0199	93-0199	
CD20	2H7	mlgG2b		57-0209		95-0209
CD27	O323	mlgG1				95-0279
CD44	IM7	rlgG2b	48-0441	57-0441	93-0441	
CD45 (LCA)	HI30	mlgG1		57-0459	93-0459	
CD45RA	HI100	mlgG2b	48-0458		93-0458	
CD45RO	UCHL1	mlgG2a				95-0457
CD62L	DREG-56	mlgG1			93-0629	
CD95	DX2	mlgG1	48-0959	57-0959		
CD117	YB5.B8	mlgG1	48-1179	57-1179		
CD123	6H6	mlgG1	48-1239			
CD127	RDR5	mlgG1		57-1278		
CD282	T2.5	mlgG1		57-9024		
CD284	HTA125	mlgG2a		57-9917		
Foxp3	236A/E7	mlgG1	48-4777	57-4777		
Foxp3	PCH101	rlgG2a		57-4776		
HLA-DR	LN3	mlgG2b			93-9956	
IFN γ	4S.B3	mlgG1		57-7319		
IL-10	JES3-9D7	rlgG1		57-7108		
IL-12/IL-23 p40/70	C8.6	mlgG1	48-7129			
TNF α	MAB11	mlgG1		57-7349		

Mouse						
Description	Clone	Isotype	eFluor™ 450	Pacific Blue®	eFluor™ 605 ^{NC}	eFluor™ 650 ^{NC}
CD3	17A2	rlgG2b	48-0032			
CD3	500A2	hamlgG		57-0033		
CD4	GK1.5	rlgG2b			93-0041	95-0041
CD4	RM4-5	rlgG2a	48-0042			
CD8a	53-6.7	rlgG2a	48-0081		93-0081	95-0081
CD11b	M1/70	rlgG2b		57-0112	93-0112	
CD11c	N418	hamlgG	48-0114			
CD16/32	93	rlgG2a	48-0161			
CD19	1D3	rlgG2a		57-0193		
CD21/CD35	4E3	rlgG2a		57-0212		
CD24	M1/69	rlgG2b	48-0242		93-0242	
CD31	390	rlgG2a			93-0311	
CD34	RAM34	rlgG2a		57-0341		
CD44	IM7	rlgG2b	48-0441	57-0441	93-0441	
CD45 (LCA)	30-F11	rlgG2b	48-0451		93-0451	



Please visit eBioscience.com as new violet laser products are launched regularly.

Description	Clone	Isotype	eFluor™ 450	Pacific Blue®	eFluor™ 605 ^{NC}	eFluor™ 650 ^{NC}
CD45R (B220)	RA3-6B2	rlgG2a	48-0452		93-0452	95-0452
CD45.1	A20	mlgG2a	48-0453			95-0453
CD62L	MEL-14	rlgG2a		57-0621	93-0621	
CD69	H1.2F3	hamlgG			93-0691	
CD73	TY/11.8	rlgG1	48-0731			
CD86	GL1	rlgG2a			93-0862	
CD90.1	HIS51	mlgG2a	48-0900	57-0900	93-0900	
CD90.2	53-2.1	rlgG2a	48-0902			95-0902
CD102	3C4	rlgG2a	48-1021			
CD105	MJ7/18	rlgG2a		57-1051		
CD122	TM-b1	rlgG2b		57-1222		
CD127	A7B34	rlgG2a		57-1271		
CD282	T2.5	mlgG1		57-9024		
CD309	Avas12a1	rlgG2a		57-5821		
F4/80	BM8	rlgG2a	48-4801			
Foxp3	FJK-16s	rlgG2a	48-5773			
IFN γ	XMG1.2	rlgG1		57-7311		
IgD	11-26c	rlgG2a	48-5993			
IgM	II/41	rlgG2a				95-5790
IL-2	JES6-5H4	rlgG2b		57-7021		
Ly-6A/E	D7	rlgG2a			93-5981	
Ly-6G	RB6-8C5	rlgG2b	48-5931			
MHC Class II (I-A/I-E)	M5/114.15.2	rlgG2b	48-5321			95-5321
NK-1.1	PK136	mlgG2a		57-5941		
TER-119	TER-119	rlgG2b	48-5921		93-5921	
TNF α	MP6-XT22	rlgG1	48-7321			
Mouse Hematopoietic Lineage Flow Cocktail	Various			88-7773		

Controls

Description	Isotype	eFluor™ 450	Pacific Blue®	eFluor™ 605 ^{NC}	eFluor™ 650 ^{NC}
IgG Isotype Control	hamlgG	48-4888	57-4888	93-4888	
IgG Isotype Control	hamlgG		57-4914		
IgG1,k Isotype Control	mlgG1	48-4714	57-4714	93-4714	95-4714
IgG2a,k Isotype Control	mlgG2a	48-4724	57-4724	93-4724	95-4724
IgG2b Isotype Control	mlgG2b	48-4732	57-4732	93-4732	95-4732
IgM Isotype Control	mlgM		57-4752		
IgG1 Isotype Control	rlgG1	48-4301	57-4301		
IgG2a Isotype Control	rlgG2a	48-4321	57-4321	93-4321	95-4321
IgG2b Isotype Control	rlgG2b	48-4031	57-4031	93-4031	95-4031
IgG2b Isotype Control	rlgG2b		57-4331		
IgM Isotype Control	rlgM		57-4341		
Streptavidin	NA	48-4317		93-4317	


Support Products

Description	Catalog No.	Description	Catalog No.
Red Blood Cell Lysis Buffer, 1X	00-4333	Brefeldin A Solution	00-4506
Flow Cytometry Staining Buffer	00-4222	Monensin Solution	00-4505
eFluor™ NC Flow Cytometry Staining Buffer	00-3222	Foxp3 Fixation/Permeabilization Concentrate	00-5123
Human Fc γ R-Binding Inhibitor	14-9161	Foxp3 Fixation/Permeabilization Diluent	00-5223
Anti-Mouse CD16/32 - Blocks Fc Binding	14-0161	Foxp3 Fixation/Permeabilization Concentrate and Diluent	00-5521
Normal Mouse Serum	24-5544	Foxp3 Staining Buffer Set	00-5523
Normal Rat Serum	24-5555	Propidium Iodide Staining Solution	00-6990
IC Fixation Buffer	00-8222	7-AAD Viability Staining Solution	00-6993



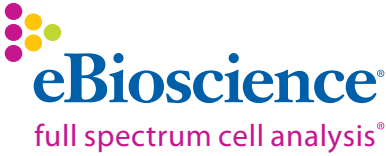
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Possess the Power of the Full Spectrum!

															
BANDPASS FILTERS:		450 / 50	530 / 30	575 / 26	605 / 40	605 / 50	610 / 20	660 / 40	660 / 20	670 / 14	695 / 40	710 / 40	780 / 60		
LASERS	Fluorochromes	UV (325-355 nm)				eFluor® 605NC	eFluor® 625NC			eFluor® 650NC					
		Violet (405 nm)	eFluor® 450				eFluor® 605NC	eFluor® 625NC			eFluor® 650NC				
		Blue (488 nm)	FITC (518 nm)		PE (575 nm)							PE-Cy5 (667 nm)	PerCP-Cy5.5 (695 nm)	PerCP-eFluor® 710	PE-Cy7 (785 nm)
			AF 488 (519 nm)												
		Yellow / Green (532-561 nm)			PE (575 nm)							PE-Cy5 (667 nm)			PE-Cy7 (785 nm)
		Red (635-655 nm)								APC (660 nm)				AF 700 (723 nm)	APC-eFluor® 780
								AF 647 (668 nm)							
								eFluor® 660							
	Proliferation	Blue (488 nm)	CFSE (521 nm)												
		Red (635-655 nm)								CPD eFluor® 670					
	Viability	UV (325-355 nm)	Calcein Blue AM (445 nm)												
		Violet (405 nm)	FVD eFluor® 450												
Calcein Violet AM (452 nm)															
Blue (488 nm)		Calcein AM (515 nm)					PI (617 nm)	7-AAD (647 nm)							
Red (635-655 nm)								FVD eFluor® 660					FVD eFluor® 780		

Note: Peak emission for eFluor® dyes is noted in the name. Peak emission for all other dyes is shown in parentheses. Before combining reagents in multicolor experiments, always refer to your specific system configuration.

Product Key: AF= Alexa Fluor®; CPD= Cell Proliferation Dye; FVD= Fixable Viability Dye; PI= Propidium Iodide



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