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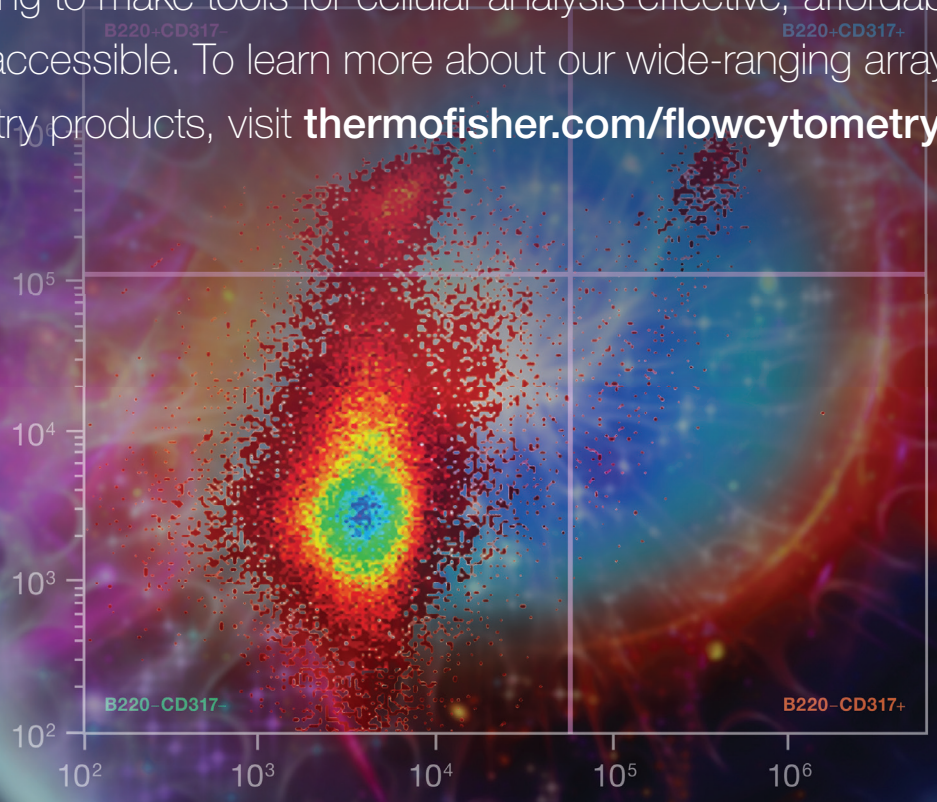
Brighter conjugates for better discrimination of dim cell populations

eBioscience Super Bright antibody conjugates for flow cytometry

ThermoFisher SCIENTIFIC

Strengthening our flow cytometry portfolio– eBioscience antibodies

At Thermo Fisher Scientific, we are committed to furthering your scientific advances by providing a comprehensive suite of products for the analysis of cells and their functions. The addition of eBioscience™ flow cytometry antibodies and reagents to the portfolio of Invitrogen™ flow cytometry reagents and instruments enables us to better provide the tools your experiments demand. Our dedicated service and support teams, and educational resources are here to help you achieve your research goals. From antibodies and functional reagents to instrumentation, Thermo Fisher Scientific is here to accelerate your research. We are focused on advancing meaningful discoveries and partnering to make tools for cellular analysis effective, affordable, and widely accessible. To learn more about our wide-ranging array of flow cytometry products, visit **thermofisher.com/flowcytometry**



Super Bright antibody conjugates

Brighter antibodies from a trusted source

Thermo Fisher Scientific continues to keep pace with flow cytomtery innovation. Invitrogen™ eBioscience™ Super Bright antibody conjugates are a valuable addition to the Invitrogen™ flow cytometry portfolio with their exceptionally bright color and photostability. These dye conjugates follow in the history of quality and innovation that has made Invitrogen™ eBioscience™ antibodies one of the most trusted names in flow cytometry research. Discover your options with the newest violet-fluorescent Super Bright conjugates from Thermo Fisher Scientific.

Super Bright antibody conjugates

The expanded use of multicolor flow cytometry spurred the development of new dyes and antibody conjugates. The Super Bright antibody conjugates are an optimal choice as they brightly fluoresce and can have lower background than competitor reagents. For cells with few expressed receptors, these bright reagents can provide the difference to detect and resolve dim populations.

Underutilized lasers, including violet, are in demand as the need for more colors and channels grows. Super Bright conjugates are an ideal addition for large, multicolor flow cytometry panels because the Super Bright dyes are polymer-based fluorophores that are excited by the violet laser (405 nm) and emit signal with high efficacy. They allow for greater utilization of the violet laser by enabling detection channels from 436 nm extended to 702 nm. This provides greater separation of channels, and in some cases, reduced compensation. Each dye is conveniently named according to their emission wavelength (Figure 1), and stability studies indicate that all Super Bright conjugates exhibit minimal loss of fluorescence when cells are exposed to formaldehyde fixative for up to three days, or when exposed overnight to ambient light. The Super Bright conjugates are a great option not only for their ability to use more color channels, but also for their compatibility with standard fluorophores, compensation beads, viability stains, and reagents.

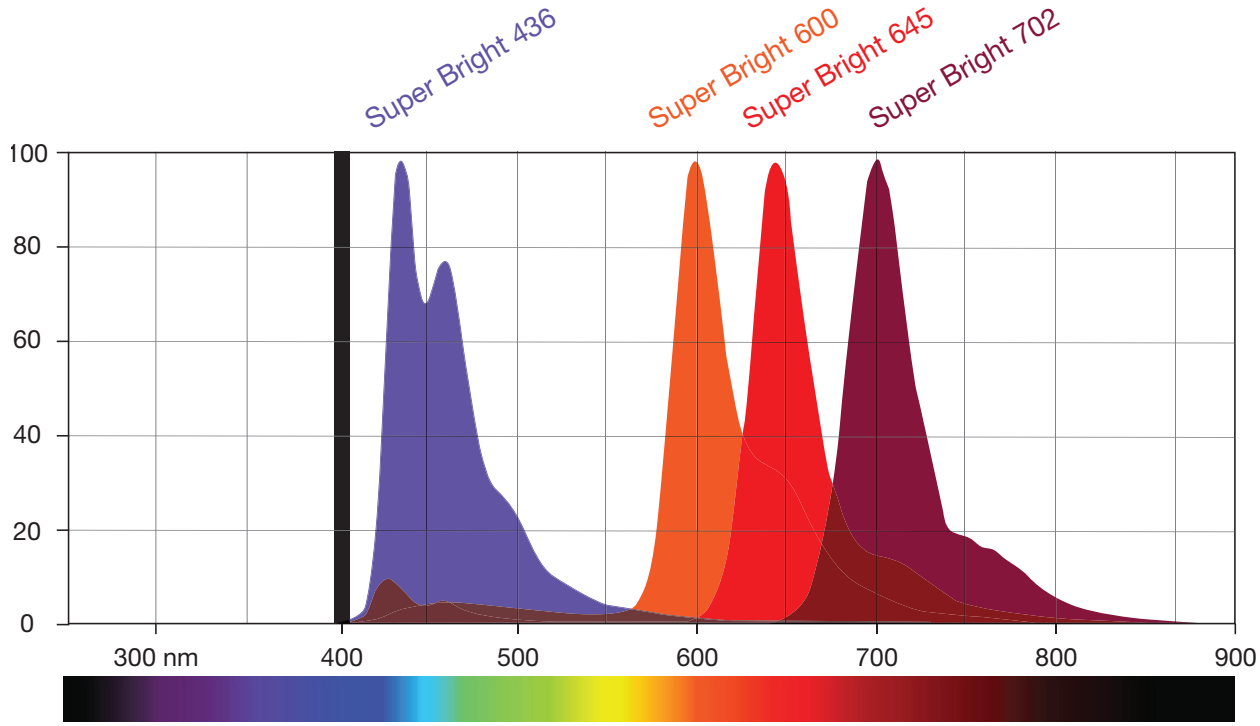


Figure 1. Emission spectra of Super Bright 436, Super Bright 600, Super Bright 645, and Super Bright 702 polymer dyes. The black bar indicates the excitation wavelength of the violet laser (405 nm).

Super Bright conjugates

Super Bright 436

Super Bright 436 antibody conjugates have an excitation maximum of 414 nm and an emission peak of 436 nm. A 450/50 bandpass filter or equivalent is recommended for detecting Super Bright 436 antibody conjugates. Conjugates of this polymer dye are significantly brighter than Invitrogen™ eFluor™ 450 dye conjugates and Pacific Blue™ dye conjugates (Figure 2A), and they are an alternative to Brilliant Violet™ 421 conjugates with similar resolution of positive and negative populations but with less background (Figure 2B—the purple Super Bright 436 curve is shifted left as compared to the orange curve) and less interfering dye–dye interaction than Brilliant Violet 421.

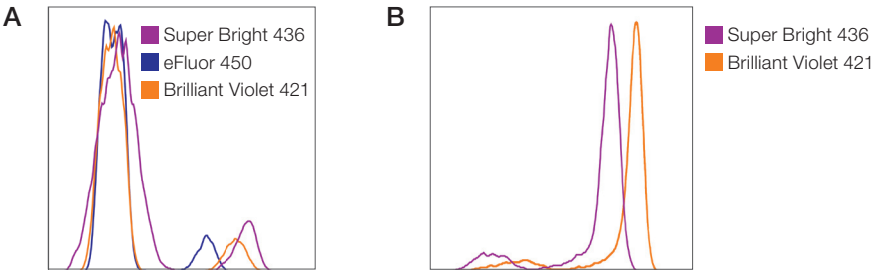


Figure 2. Fluorescence intensity for Super Bright 436 dye conjugates compared to Brilliant Violet 421 conjugates. (A) Human peripheral blood cells were stained with CD19 antibody conjugated to either Super Bright 436 (purple, Cat. No. 62-0199-42), eFluor 450 dye (blue, Cat. No. 48-0199-42), or Brilliant Violet 421 dye (orange). **(B)** Human peripheral blood cells were stained with CD27 antibody conjugated to either Super Bright 436 (purple, Cat. No. 62-0279-42) or Brilliant Violet 421 dye (orange).

Super Bright 600

Super Bright 600 is a tandem dye consisting of Super Bright 436 and an acceptor dye that emits a fluorescence signal at 600 nm. Antibody conjugates can be detected using a 610/20 bandpass filter or equivalent, and conjugates of this tandem polymer are an alternative to Brilliant Violet™ 605 conjugates (Figure 3A) with equivalent brightness. As with all Super Bright conjugates, Super Bright 600 conjugates are stable for up to three days when stored in a formaldehyde fixative solution and protected from exposure to light (Figure 3B).

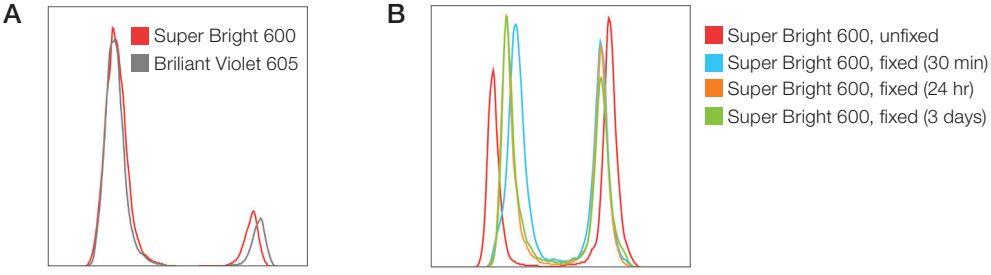


Figure 3. Staining performance and post-fixation stability. (A) Comparison of mouse splenocytes stained with CD8a antibody conjugated to either Super Bright 600 (red, Cat. No. 63-0081-82) or Brilliant Violet 605 conjugate (gray), at the same antibody concentration. **(B)** Mouse splenocytes were stained with CD45R antibody conjugated to Super Bright 600 (Cat. No. 63-0452-82) and either left unfixed (red), or were fixed in Invitrogen™ eBioscience™ IC Fixation Buffer (Cat. No. 00-8222-49) for 30 minutes (blue), 24 hours (orange), or 3 days (green).

Super Bright 645

Super Bright 645 is a tandem dye consisting of Super Bright 436 and an acceptor dye that has an emission peak of 645 nm. It can be detected using a 660/20 bandpass filter or equivalent. Antibody conjugates of this tandem polymer dye are comparable, and sometimes superior in brightness to Brilliant Violet™ 650 antibody conjugates (Figure 4) with less spillover into other violet channels.

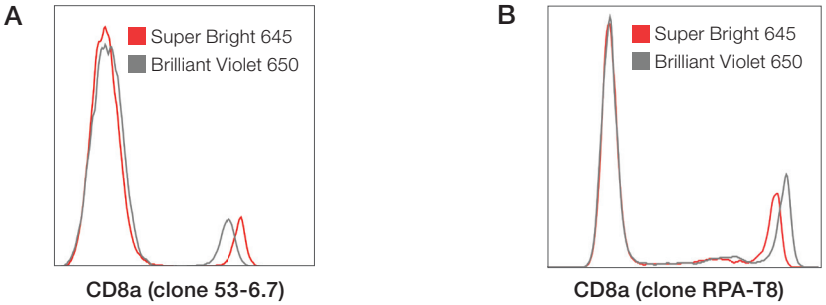


Figure 4. Fluorescence intensity comparison of Super Bright 645 conjugates and Brilliant Violet 650 conjugates. (A) Mouse splenocytes stained with anti-CD8a conjugated to Super Bright 645 (red, Cat. No. 64-0081-82) or Brilliant Violet 650 conjugate (gray), at the same concentration of antibody. **(B)** Human peripheral blood cells stained with anti-CD8a conjugated to Super Bright 645 (red, Cat. No. 64-0088-42) or Brilliant Violet 650 conjugate (gray), using the same concentration of antibody.

Super Bright 702

Super Bright 702 tandem dye, consists of Super Bright 436 and an acceptor dye that has an emission peak of 702 nm. Conjugates can be detected using a 710/50 bandpass filter or equivalent, and Super Bright 702–conjugated antibodies are similar in brightness to Brilliant Violet™ 711 conjugates (Figure 5) with reduced compensation and less spillover into the Brilliant Violet™ 786 channel and certain blue laser channels.

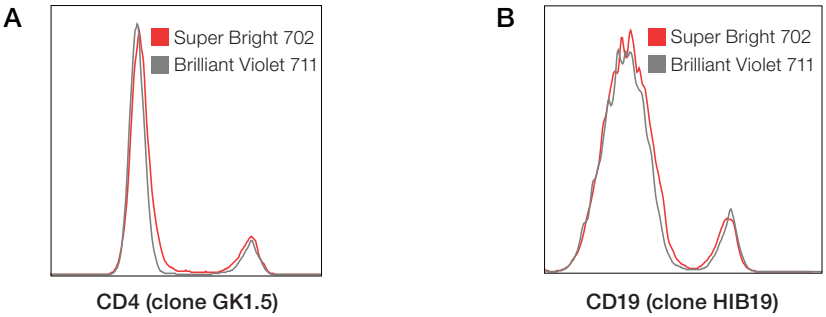


Figure 5. Fluorescence intensity comparison of Super Bright 702 conjugates to Brilliant Violet 711 conjugates. (A) Mouse splenocytes stained with anti-CD4 conjugated to Super Bright 702 (red) or Brilliant Violet 711 conjugate (gray), at the same concentration of antibody. **(B)** Human peripheral blood cells stained with anti-CD19 conjugated to Super Bright 702 (red) or Brilliant Violet 711 conjugate (gray), using the same concentration of antibody.

Multiplexing made easy

Super Bright antibody conjugates can be utilized in flow cytometry applications without adjusting standard workflows.

Advantages of using Super Bright conjugates

The difficult-to-identify cells often have non-abundant markers, or share many markers with other cell types. Super Bright conjugates can be used to identify these cells by providing increased detection of low-abundance antigens, while also allowing for more channels for analysis off the violet laser. Super Bright dye technology requires less compensation, and has less dye–dye interactions than comparable products.

Super Bright Staining Buffer

Invitrogen™ eBioscience™ Super Bright Staining Buffer (Cat. No. SB-4400-42) is recommended when combining two or more Super Bright conjugates in the same flow cytometry panel. The buffer minimizes any nonspecific interactions that may occur between polymer-based dye conjugates (Figure 6). The Super Bright Staining Buffer is convenient in a ready-to-use 5 µL/test, and can be used in multicolor panels when Super Bright conjugates are combined with other polymer dye conjugates including Brilliant Violet conjugates (Figure 7).

Super Bright conjugate compatibility

Fully compatible with Invitrogen™ UltraComp eBeads™ microspheres for compensation (Figure 8, Cat. No. 01-2222-42), viability stains, and other reagents commonly used in flow cytometry, Super Bright antibody conjugates easily fit into any flow cytometry panel (Figure 9). Super Bright technology empowers researchers to answer complex cell biology questions in less time, with lower cell numbers and fewer samples.

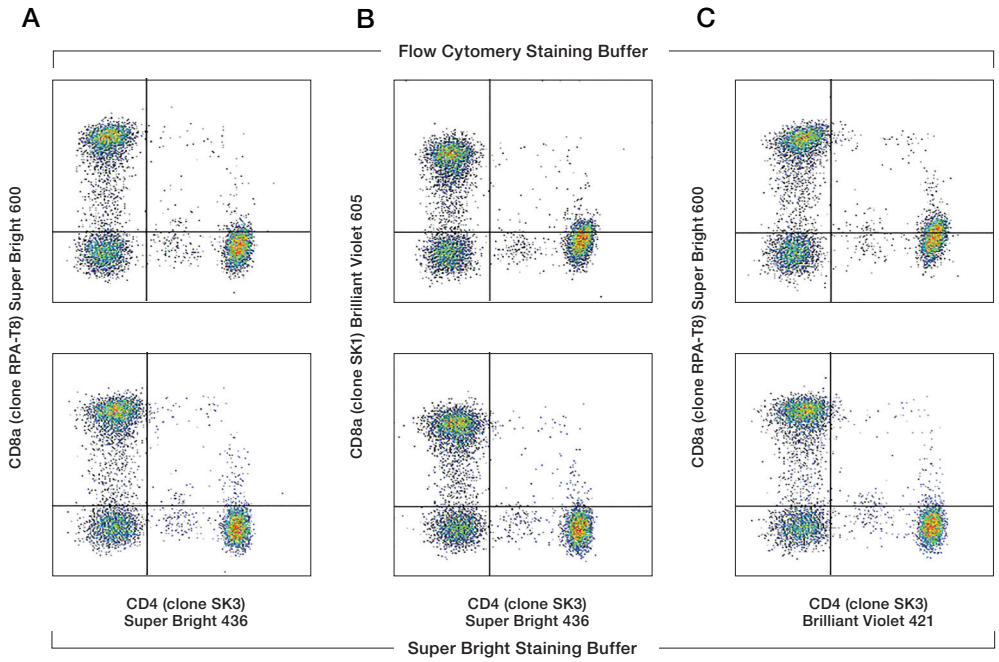


Figure 6. Super Bright Staining Buffer mitigates nonspecific polymer dye interactions. The top plots depict use of the Invitrogen™ eBioscience™ Flow Cytometry Staining Buffer (Cat. No. 00-4222-26), whereas the bottom plots show use of the Super Bright Staining Buffer (Cat. No. SB-4400-42). Flow Cytometry Staining Buffer or the Super Bright staining buffer was added to human peripheral blood cells prior to staining with **(A)** Anti-CD8a Super Bright 600 (Cat. No. 63-0088-42), and Anti-CD4 Super Bright 436 (Cat. No. 62-0047-42), **(B)** Anti-CD8 Brilliant Violet 605 and Anti-CD4 Super Bright 436 (Cat. No. 62-0047-42), or **(C)** Anti-CD8a Super Bright 600 (Cat. No. 63-0088-42) and Anti-CD4 Brilliant Violet 421.

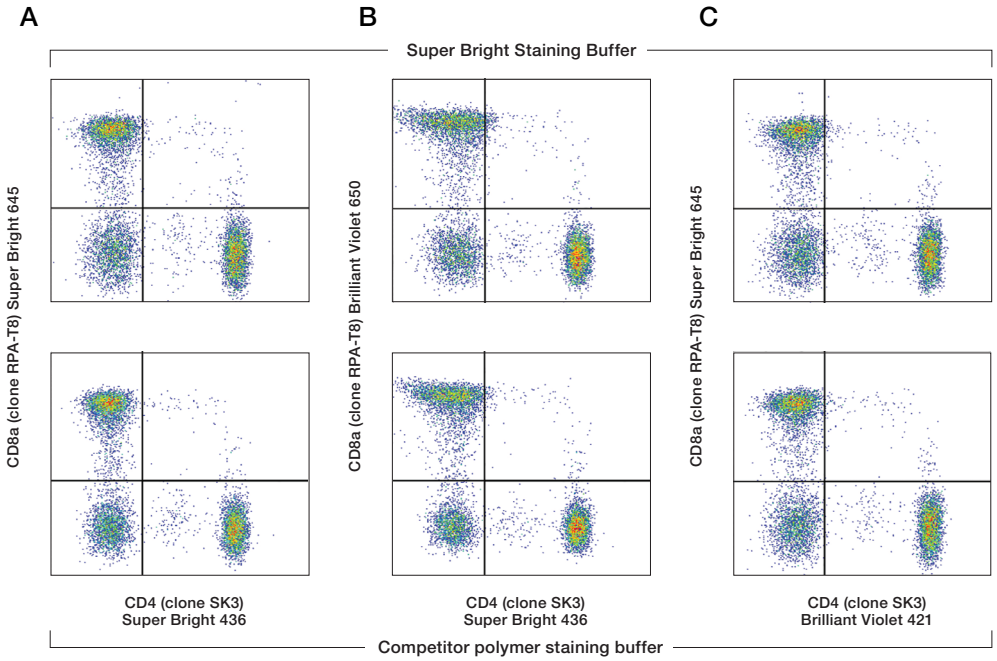


Figure 7. Super Bright Staining Buffer comparison to a polymer staining buffer from another supplier. Super Bright Staining Buffer (top plot, Cat. No. SB-4400-42) or a polymer staining buffer from another supplier (bottom plot) was added to human peripheral blood cells prior to staining with **(A)** Anti-CD8a Super Bright 645 (Cat. No. 64-0088-42) and Anti-CD4 Super Bright 436 (Cat. No. 62-0047-42) **(B)** Anti-CD8a Brilliant Violet 650 and Anti-CD4 Super Bright 436 (Cat. No. 62-0047-42), or **(C)** Anti-CD8a Super Bright 645 (Cat. No. 64-0088-42) and Anti-CD4 Brilliant Violet 421.

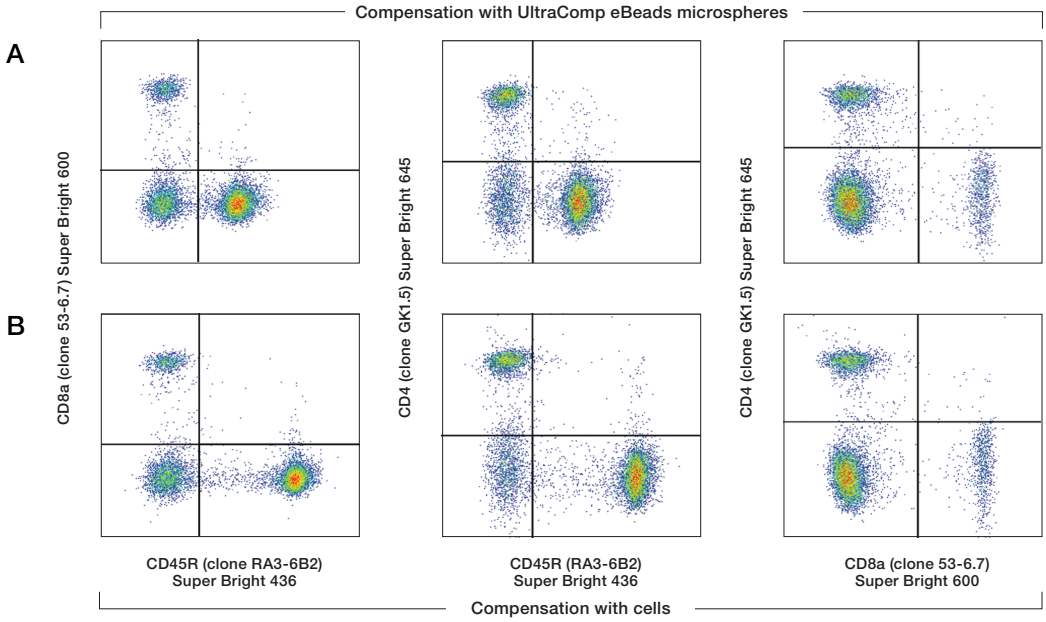


Figure 8. Super Bright antibody conjugates are compatible with UltraComp eBeads microspheres. Mouse splenocytes were stained with a three-color panel comprised of Anti-CD45R/B220 Super Bright 436, Anti-CD8a Super Bright 600 (Cat. No. 63-0081-82), and Anti-CD4 Super Bright 645. Compensation was set using **(A)** UltraComp eBeads microspheres (top row Cat. No. 01-2222-42) or **(B)** with cells (bottom row). Compensation values with beads were similar to single-color stained cells (not shown).

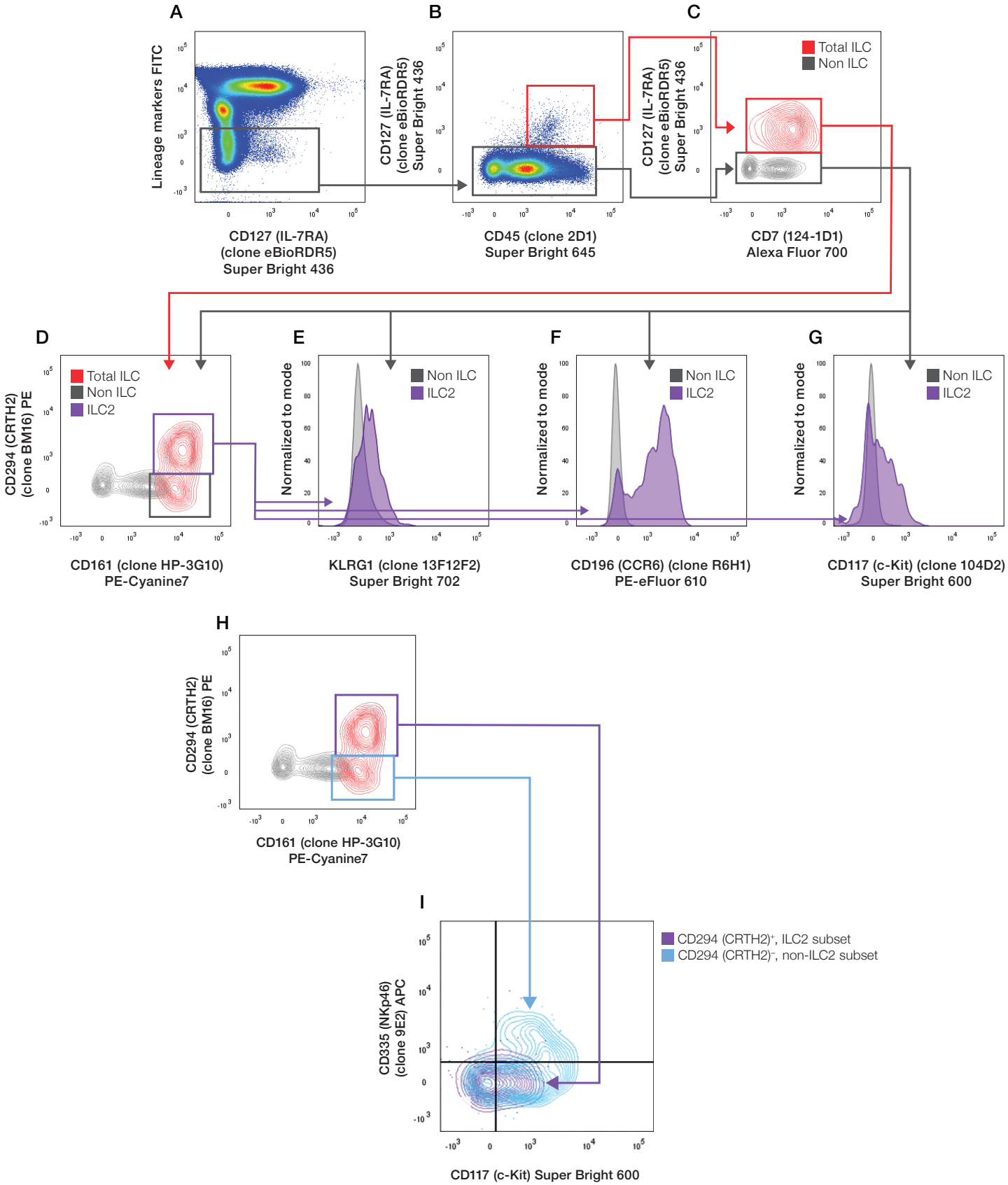


Figure 9. 10-color ILC2 subset panel. Normal human PBMCs were surface-stained in the presence of Super Bright Staining Buffer (Cat. No. SB-4400-42) at optimal concentrations for the indicated surface markers. **(A)** Gated on live cells, this plot shows the lineage markers used as a FITC dump channel (CD3 (clone UCHT1), CD4 (clone SK3), CD8a (clone RPA-T8), CD11b (clone ICRF44), and CD19 (clone HIB19) vs. CD127 (IL-7RA) (clone eBioRDR5) Super Bright 436 (Cat. No. 62-1278-42) stained cells. Since ILC2 subsets are negative for all five of these markers, all CD127$^-$ and lineage-positive cells can be eliminated from further analysis. **(B-G)** Gating strategy is shown for all lineage$^-$ targets to highlight the ILC2 population. **(H, I)** The CD294 (CRTH2)$^+$ population is identified.

Table 1. Selection guide for anti-human Super Bright antibody conjugates.*

Human	Clone	Super Bright 436	Super Bright 600	Super Bright 645	Super Bright 702
CD3	OKT3		●	●	●
CD3	UCHT1	●			
CD4	SK3 (SK-3)	●	●	●	●
CD5	UCHT2	●			
CD8a	RPA-T8	○	●	●	●
CD11b	ICRF44	●	●		
CD11c	3.9		●	●	●
CD14	61D3		●	●	●
CD16	eBioCB16 (CB16)	○	●	●	
CD16	3G8				●
CD19	SJ25C1		●		
CD19	HIB19	●	○	●	●
CD20	2H7	●	●	●	
CD23	EBVCS2	●			
CD25	BC96	●	●	●	●
CD27	O323	●	●	○	
CD34	4H11		●	●	
CD39	eBioA1 (A1)	●			
CD44	IM7	●	○	●	●
CD45	2D1		●	●	○
CD45R (B220)	RA3-6B2		●	●	
CD45RA	HI100		○	●	●
CD45RO	UCHL1		●	●	
CD56 (NCAM)	TULY56	○	●	●	●
CD83	HB15e	●			
CD86 (B7-2)	IT2.2	●			
CD90 (Thy-1)	eBio5E10 (5E10)	●			
CD117 (c-Kit)	104D2		●		
CD123	6H6	●	●	○	
CD127	eBioRDR5	●	●	●	
CD133	TMP4		●		
CD137	4B4 (4B4-1)	●			
CD138 (Syndecan-1)	DL-101		●		
CD162 (PSGL-1)	FLEG	●			
CD183 (CXCR3)	CEW33D	●			
CD184 (CXCR4)	12G5	●			
CD196 (CCR6)	R6H1	●	●		
CD223 (LAG-3)	3DS223H	●	●		
CD278 (ICOS)	ISA-3	●			
CD335 (NKp46)	9E2	●	●		
CD366 (TIM3)	F38-2E2	●	○	○	
HLA-DR	LN3		●	○	
KLRG1	13F12F2				●
IgM	SA-DA4	○	●		

* ●●●●: Filled circles represent antibody conjugates already released.
○○○○: Open circles represent antibody conjugates that will be avaliable soon.

To learn more about these polymer dyes and search the expanding portfolio of Super Bright antibody conjugates, please visit **thermofisher.com/superbright**

Table 2. Selection guide for anti-mouse Super Bright antibody conjugates.*

Mouse	Clone	Super Bright 436	Super Bright 600	Super Bright 645	Super Bright 702
CD4	RM4-5	○	●	●	●
CD4	GK1.5	●			
CD8a	53-6.7	○	●	●	●
CD11b	M1/70	●	●	●	●
CD19	eBio1D3 (1D3)		●	○	
CD39	24DMS1	●	○		
CD44	IM7	●	○	●	●
CD45	30-F11		●	●	●
CD45R/B220	RA3-6B2		●	●	
CD45.1	A20			●	●
CD45.2	104		●	●	
CD62L	MEL-14		●		
CD80 (B7-1)	16-10A1		●	●	
CD86 (B7-2)	GL1		●	●	
CD93 (AA4.1)	AA4.1	●			
CD95 (APO-1/fas)	15A7	●			
CD117 (c-Kit)	2B8	●	●	●	
CD140a	APA5	●			
CD170 (siglec F)	1RNM44N	●	●	○	○
CD205	205yekta	●			
CD274 (PD-L1, B7-H1)	MIH5	●			
Ly-6A/E (Sca-1)	D7	●	●		
Ly-6G (Gr-1)	RB6-8C5		●	●	
MHC II (I-A/I-E)	M5/114.15.2			●	
NK1.1	PK136	●	●	●	

Table 3. Selection guide for Super Bright isotype controls.*

Isotype controls	Clone	Super Bright 436	Super Bright 600	Super Bright 645	Super Bright 702
Mouse IgG1 Kappa	P3.6.2.8.1	●	●	●	●
Mouse IgG2a Kappa	eBM2a		●	●	●
Mouse IgG2b Kappa	eBMG2b	●	●		●
Rat IgG2a Kappa	eBR2a	●	●	●	●
Rat IgG2b Kappa	eB149/10H5	●	●	●	●

* ●●●●: Filled circles represent antibody conjugates already released.
○○○○: Open circles represent antibody conjugates that will be avaliable soon.

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High-performance flow cytometry

The Attune NxT Flow Cytometer—
6 fluorescence channels off the violet laser will be available soon

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- **Investigate**—in less time, with less sample and with less clogging—leveraging the power of acoustic focusing

The Invitrogen™ Attune™ NxT Flow Cytometer, introduced with up to 4 lasers and 16 parameters of detection, will soon be available with 6 fluorescence detectors for the violet laser (Table 4). The 6-channel violet laser configuration will accommodate the Super Bright antibody conjugates along with other commercially available violet-excitable fluorophores (Table 5). Learn more about how the Attune NxT Flow Cytometer makes life easier by simplifying sample prep, allowing the exploration of more sample types (including tumor samples) without clogging, and maximizing your throughput with walk-away ease at **thermofisher.com/attune**

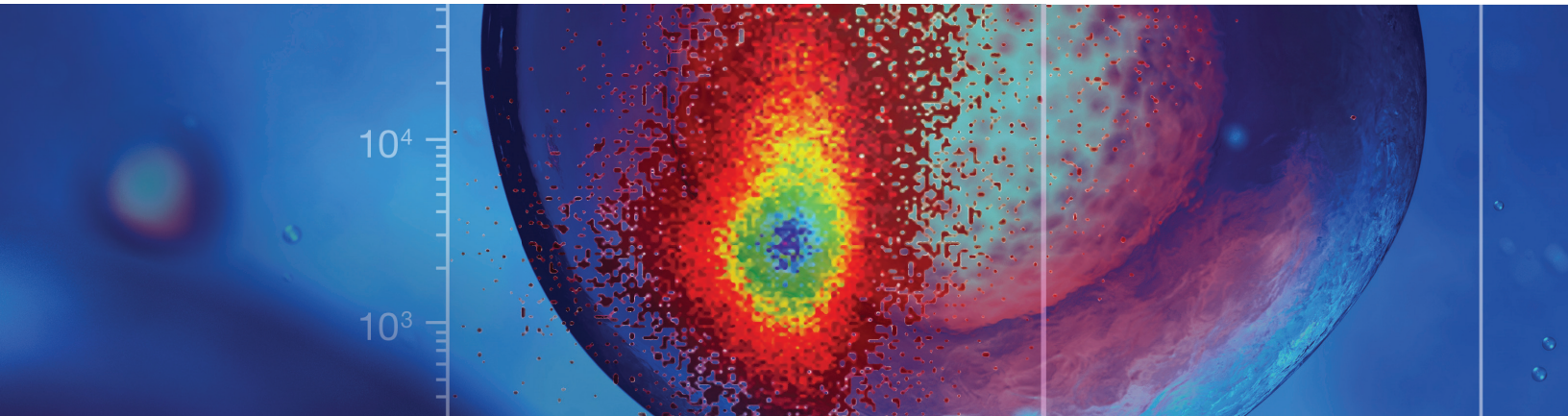
Table 4. Attune NxT Flow Cytometer configuration using 6 fluorescence detectors for the violet laser.

Laser	Fluorescence detectors		
	2-laser	3-laser	4-laser
Violet, 405 nm	6	6	6
Blue, 488 nm	2	2	2
Yellow, 561 nm	NA	NA	3
Red, 637 nm	NA	3	3
Total fluorescence detectors available	8	11	14
Total parameters per configuration (includes FSC and SSC)*	10	13	16

* FSC: forward scatter, SSC: side scatter.

Table 5. Fluorophore guidelines for the 6 fluorescence detectors off the violet laser in the Attune NxT Flow Cytometer.

Detector	Bandpass (nm)	Fluorophores
VL1	450/40	Super Bright 436, Brilliant Violet 421, eFluor 450, Pacific Blue, BD Horizon™ V450, VioBlue
VL2	525/50	eFluor 506, Brilliant Violet 510, Pacific Green, BD Horizon™ V500, VioGreen
VL3	610/20	Super Bright 600, Brilliant Violet 605, Pacific Orange
VL4	660/20	Super Bright 645, Brilliant Violet 650
VL5	710/50	Super Bright 702, Brilliant Violet 711
VL6	780/60	Brilliant Violet 786



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Reference guides

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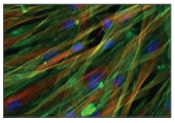

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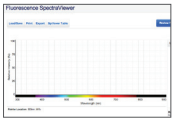

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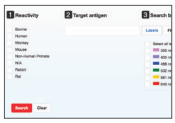

Flow Cytometry Panel Design Tool
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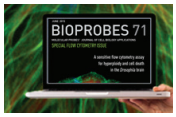

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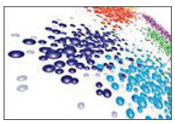

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