

BD Horizon Brilliant™ Blue 515 Reagents

Features

- Provide excellent population resolution, especially for dim populations
- Have less spillover into the PE channel compared to FITC
- Offer a significantly brighter alternative to FITC

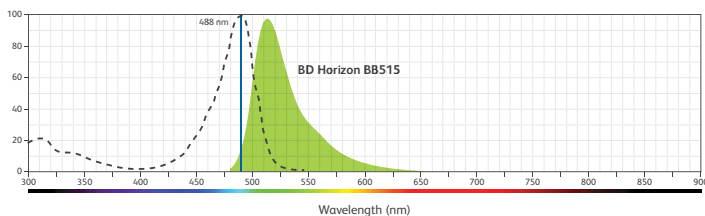


Figure 1. Absorption and emission spectra
Ex Max: 490, Em Max: 515

	Stain Index		
	BB515	FITC	Alexa Fluor® 488
Hu CD3	302	43	81
Hu CD4	174	47	58
Hu CD19	85	16	15
Ms CD8α	86	24	50
Ms CD11b	68	15	26

Table 1. BD Horizon BB515, Alexa Fluor® 488, and FITC reagents of the same clone run side by side to compare the stain index

BD Horizon Brilliant™ Blue 515 (BB515) was developed exclusively by BD Biosciences as an additional bright dye to better resolve dim populations. This dye is brighter than FITC and has less spillover into the PE channel, making it more optimal for multicolor flow cytometry.

An innovative dye for the FITC channel

Developed using technology from Sirigen, BD Horizon BB515 is significantly brighter than FITC and has less spillover into neighboring channels (Table 1 and 2, Figure 2). The dye is optimal for dimmer markers, such as CD25, for which better resolution improves the quality of a panel. CD25 FITC or CD25 BB515 was used to identify regulatory T cells (Tregs) in a panel including CD4 APC, CD127 PE and CD3 PerCP-Cy™5.5. While both panels resolve the Treg population, the panel including CD25 BB515 shows significantly better separation of the CD25-positive cells from the CD25-negative cells (Figure 3). FoxP3 transcripts have been identified in CD4⁺CD25^{hi}CD127^{dim} cells, and optimal resolution of these markers is necessary to identify the various subsets within the panel.^{1,2} The FITC format is too dim to fully resolve the CD25 bright cells from the intermediates. However, the brightness of BD Horizon BB515 provides excellent resolution with optimal identification of the Treg population. This provides more flexibility in panel design; previously the FITC channel had to be reserved for highly expressed markers. With the introduction of the BD Horizon BB515 format, researchers can now use this channel to optimally resolve both dimly and highly expressed markers.



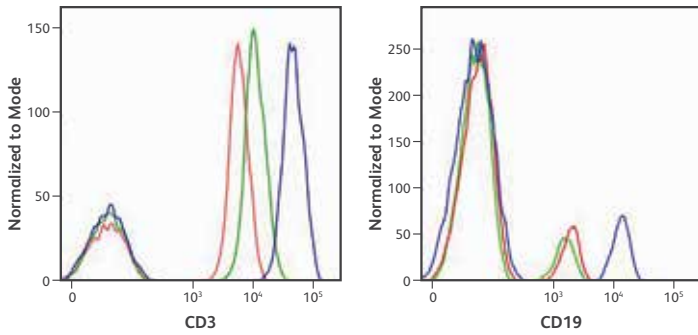


Figure 2. Lysed whole blood stained with Hu CD3 or CD19 FITC (red), BB515 (blue), or Alexa Fluor® 488 (green). Data shown was gated on lymphocytes.

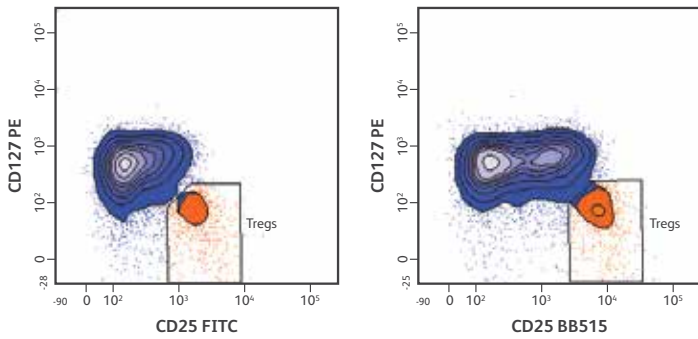


Figure 3. Lysed whole blood stained with Hu CD4 APC, CD127 PE, CD3 PerCP-Cy5.5 and CD25 FITC or CD25 BB515, and analyzed on a BD FACSVerser flow cytometer. Data shown was gated on CD4⁺CD3⁺ lymphocytes.

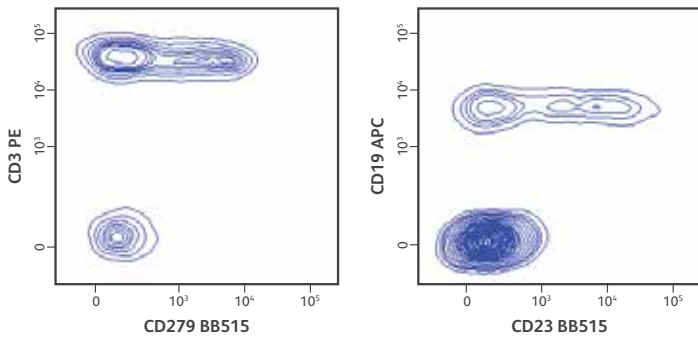


Figure 4. Example data of BB515 staining to resolve dim populations
 A. Lysed whole blood was stained with Hu CD279 BB515 and CD3 PE. Data shown was gated on lymphocytes.
 B. Lysed whole blood was stained with Hu CD23 BB515 and CD19 APC. Data shown was gated on lymphocytes.

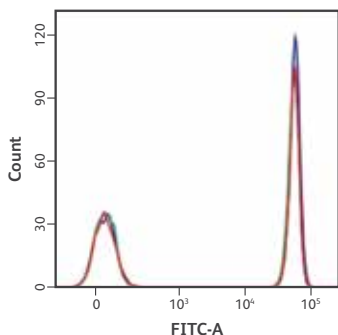


Figure 5. Data from three lots of BB515 conjugates
 Three different lots of BB515 dye were conjugated to Hu CD4. The conjugates were run side by side, and staining is shown on lymphocytes (Lot 1: Blue line, Lot 2: Red line, Lot 3: Green line). The spillover values into various channels were also evaluated for consistency (Table 3).

With a peak excitation at 490 nm and emission at 515 nm, BD Horizon BB515 can be excited by the blue laser and detected in a standard FITC filter (for example, 530/30 nm). BD Horizon BB515 can be used to replace FITC or Alexa Fluor® 488 conjugates. The dye can be used on any BD FACS™ brand flow cytometer equipped with a blue laser, including the BD Accuri™ C6, BD FACSCalibur™, BD FACSVerser™, BD FACSCanto™ II, BD LSRFortessa™, BD FACSARIA™, BD Influx™ and BD FACSJazz™.

As with all BD reagents, a high level of quality ensures reproducible results through minimal lot-to-lot variability. During development, three lots of the BB515 dye were tested to ensure lot-to-lot consistency. The BD Horizon BB515 reagents have minimal lot-to-lot variability and excellent consistency. (Figure 5).

Compatible with standard surface and intracellular staining protocols

BD Horizon BB515 is compatible with standard buffers used in surface and intracellular staining protocols. These reagents also demonstrate compatibility with paraformaldehyde-based fixatives and both EDTA and heparin blood collection tubes.

	Spillover into		
	BV510	PE	PE-CF594
Hu CD4 BB515	2%	20%	6%
Hu CD4 FITC	6%	27%	9%

Table 2. Spillover into various detectors compared between BD Horizon BB515 and FITC

Whole blood samples stained with human CD4 BB515 or FITC were analyzed on a BD LSRFortessa system, and spillover was measured in the BV510, PE and PE-CF594 detectors. This table is meant to show a relative comparison between the dyes, since spillover values obtained can vary depending on the filter used and PMT voltage.

	BB515 spillover into various channels			
CD4 BB515 lot	BV510	PE	PE-CF594	APC
Lot 1	2%	20%	6%	0%
Lot 2	2%	20%	6%	0%
Lot 3	2%	20%	6%	0%

Table 3. Spillover into various detectors compared between three lots of BD Horizon BB515

A selection of BD Horizon BB515 research reagents

Check bdbiosciences.com/colors for a complete list of products.

Ordering information					
Description	React.	Clone	Isotype	Size	Cat. No.
CD4	Human	RPA-T4	Ms IgG ₁ , κ	25 Tests	564420
				100 Tests	564419
CD11b	Human	ICRF44	Ms IgG ₁ , κ	25 Tests	564518
				100 Tests	564517
CD19	Human	HIB19	Ms IgG ₁ , κ	25 Tests	564457
				100 Tests	564456
CD23	Human	M-L233	Ms IgG ₁ , κ	100 Tests	564555
CD25	Human	2A3	Ms IgG ₁ , κ	25 Tests	564468
				100 Tests	564467
CD38	Human	HIT2	Ms IgG ₁ , κ	25 Tests	564499
				100 Tests	564498
CD56	Human	B159	Ms IgG ₁ , κ	25 Tests	564489
				100 Tests	564488
CD132	Human	TUGh4	Rat IgG _{2b} , κ	50 Tests	564528
CD279	Human	EH12.1	Ms IgG ₁ , κ	50 Tests	564494
CCR10	Human	1B5	Ms IgG _{2a} , κ	25 µg	564769
CD8a	Mouse	53-6.7	Rat IgG _{2a} , κ	25 µg	564459
				0.1 mg	564422
CD11b	Mouse	M1/70	Rat IgG _{2b} , κ	25 µg	564455
				0.1 mg	564454
CD25	Mouse	PC61	Rat IgG ₁ , λ	25 µg	564458
				0.1 mg	564424
CD62L	Mouse	MEL-14	Rat IgG _{2a} , κ	0.1 mg	565261
CD93	Mouse	AA4.1	Rat IgG _{2b} , κ	50 µg	564700
CD117	Mouse	2B8	Rat IgG _{2b} , κ	0.1 mg	564481
CD138	Mouse	281-2	Rat IgG _{2a} , κ	50 µg	564511
CD223	Mouse	C9B7W	Rat IgG ₁ , κ	50 µg	564672
Streptavidin	–	–	–	0.1 mg	564453

References

1. Liu W, Putnam A, Xu-yu Z, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4⁺ T reg cells. *J Exp Med.* 2006;203:1701-1711.
2. Seddiki N, Santner-Nanan B, Martinson J, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med.* 2006;203:1693-1700.

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Alexa Fluor® is a registered trademark of Life Technologies Corporation.

Cy™ is a trademark of GE Healthcare. Cy™ dyes are subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and are made and sold under license from GE Healthcare only for research and in vitro diagnostic use. Any other use requires a commercial sublicense from GE Healthcare, 800 Centennial Avenue, Piscataway, NJ 08855-1327, USA.

Trademarks are the property of their respective owners.

23-16347-02

BD Life Sciences, San Jose, CA, 95131, USA

bdbiosciences.com

