BD Horizon Brilliant™ Blue 515 Reagents

Features

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

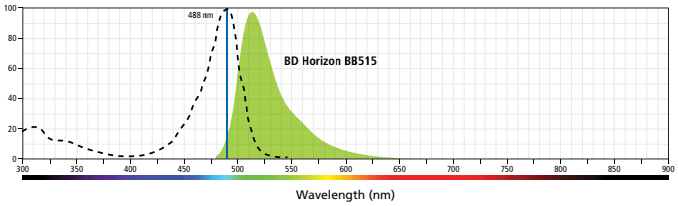

Figure 1. Absorption and emission spectra.
Ex Max: 490, Em Max: 515

	Stain Index		
	BB515	**FITC**	**Alexa Fluor® 488**
Hu CD3	302	43	81
Hu CD4	174	47	58
Hu CD19	85	16	15
Ms CD8a	86	24	50
Ms CD11b	68	15	26

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

	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.

BD Horizon Brilliant™ Blue 515 (BB515) was developed exclusively by BD Biosciences as a brighter alternative to FITC. Compared to FITC, this dye also 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 up to seven times 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.

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) (Figure 1). 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 FACSVerse™, BD FACSCanto™ II, BD LSRFortessa™, BD FACSAria™, BD Influx™, and BD FACSJazz™.

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.

Tools to optimize setup, selection, and performance

To help advance the use of multicolor flow cytometry, BD Biosciences offers a growing library of tools and resources relevant to both experienced researchers and those new to multicolor panel design (bdbiosciences.com/colors). In addition to this online resource, BD Biosciences offers one-on-one technical application support as part of our comprehensive customer services.

Fisher Scientific

Visit **fishersci.com/bdbiosciences** for more information.

BD

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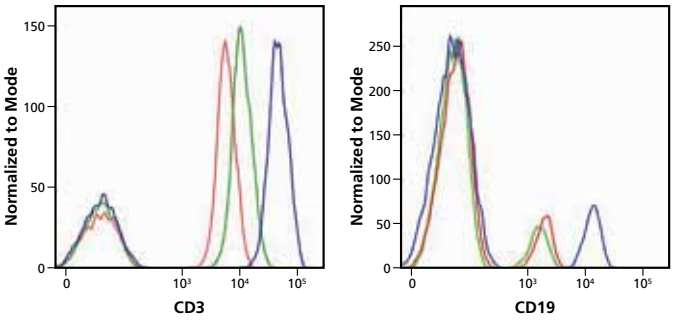

Figure 2. Lysed whole blood stained with Hu CD3 or CD19 FITC (red), BB515 (blue), or Alexa Fluor® 488 (green).
Data shown was gated on lymphocytes.

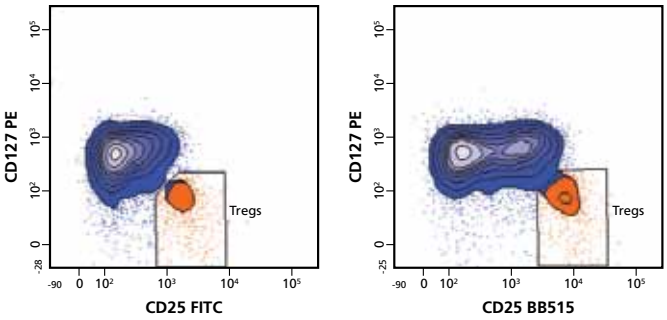

Figure 3. Whole blood was stained with Hu CD4 APC, CD127 PE, CD3 PerCP-Cy5.5, and CD25 FITC or CD25 BB515, and analyzed on a BD FACSVerse flow cytometer.
Data shown was gated on $CD4^+CD3^+$ lymphocytes.

A selection of BD Horizon BB515 Research Reagents

Check **fishersci.com/bdbiosciences** for a complete list of products.

Description	React.	Clone	Isotype	Size	Fisher Sci Cat. No.
CD4	Human	RPA-T4	Ms IgG_1, κ	25 Tests	**BDB564420**
				100 Tests	**BDB564419**
CD19	Human	HIB19	Ms IgG_1, κ	25 Tests	**BDB564457**
				100 Tests	**BDB564456**
CD25	Human	2A3	Ms IgG_1, κ	25 Tests	**BDB564468**
				100 Tests	**BDB564467**
CD38	Human	HIT2	Ms IgG_1, κ	25 Tests	**BDB564499**
				100 Tests	**BDB564498**
CD56	Human	B159	Ms IgG_1, κ	25 Tests	**BDB564489**
				100 Tests	**BDB564488**
CD8a	Mouse	53-6.7	Rat IgG_{2a}, κ	25 µg	**BDB564459**
				0.1 mg	**BDB564422**
CD11b	Mouse	M1/70	Rat IgG_{2b}, κ	25 µg	**BDB564455**
				0.1 mg	**BDB564454**
CD25	Mouse	PC61	Rat IgG_1, λ	25 µg	**BDB564458**
				0.1 mg	**BDB564424**
Streptavidin	–	–	–	0.1 mg	**BDB564453**
Ms IgG1	–	X40	–	0.1 mg	**BDB564416**
Rat IgG2a, κ	–	R35-95	–	0.1 mg	**BDB564418**
Rat IgG2b, κ	–	R35-38	–	0.1 mg	**BDB564421**
Rat IgG1, λ	–	A110-1	–	0.1 mg	**BDB564417**

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.

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