BD Horizon Brilliant™ Blue Reagents

Features

- Excellent resolution of dim populations
- Spillover advantages to optimize panel design
- More bright choices for multicolor panel design

BD Horizon Brilliant™ Blue dyes were exclusively developed by BD Biosciences as brighter options for the blue laser to better resolve dim populations. BD Horizon™ BB515 is a brighter alternative to FITC, and BD Horizon™ BB700 is a brighter alternative to PerCP-Cy™5.5. These channels were typically reserved for highly expressed markers. With the introduction of BB515 and BB700, researchers can now use these channels to optimally resolve both dimly and highly expressed markers.

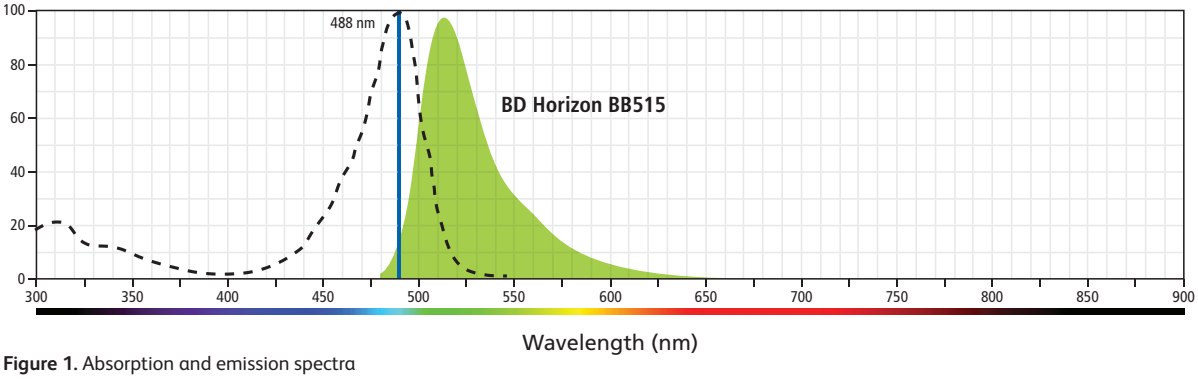

Figure 1. Absorption and emission spectra
Ex Max: 490, Em Max: 515

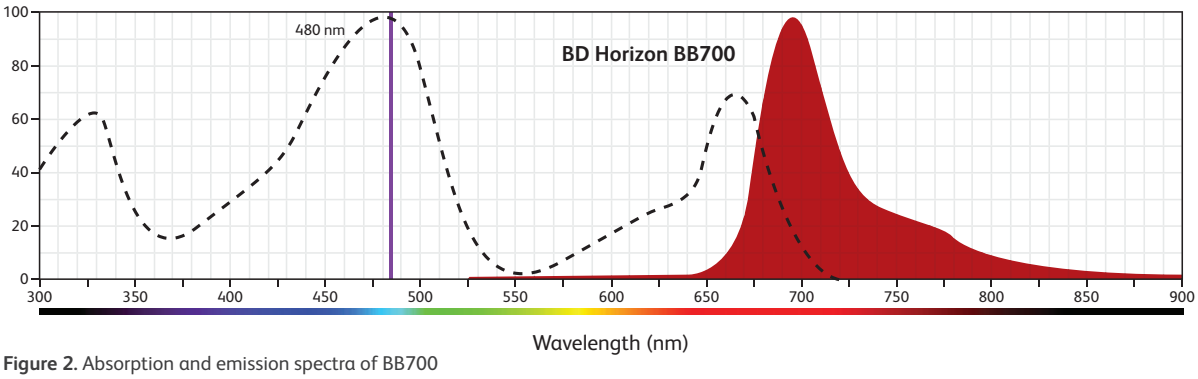

Figure 2. Absorption and emission spectra of BB700
Ex Max: 485 nm, Em Max: 693 nm

BD

BD Horizon Brilliant™ Blue 515

BB515 is significantly brighter than FITC and has less spillover into neighboring channels (Table 1 and 2, Figure 3). 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-Cy5.5. 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 4).

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.

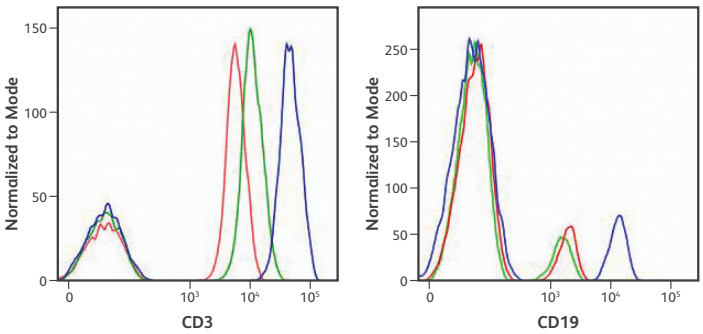

Figure 3. Lysed whole blood stained with Hu CD3 or CD19 FITC (red), BB515 (blue), or Alexa Fluor® 488 (green)
Data shown was gated on lymphocytes.

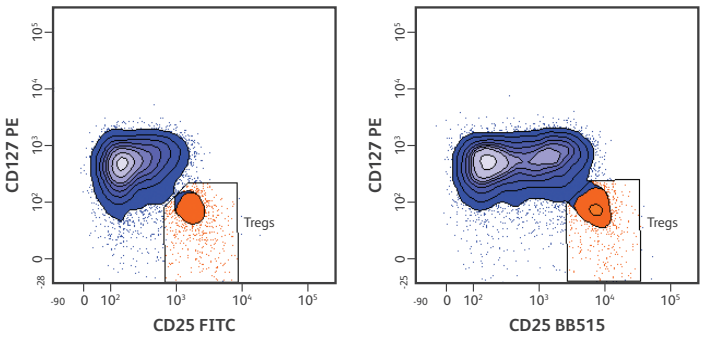

Figure 4. Lysed whole blood stained with Hu CD4 APC, CD127 PE, CD3 PerCP-Cy5.5 and CD25 FITC or CD25 BB515
Data shown was gated on $CD4^+CD3^+$ lymphocytes.

	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 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 comparison of 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 photomultiplier tube (PMT) voltage.

BD Horizon Brilliant™ Blue 700

BB700 was developed as a brighter alternative to PerCP-Cy5.5, making it better suited for resolving dim populations (Figure 5). Figure 5c shows how a dimmer dye such as PerCP-Cy5.5 could underestimate the CD279 expression, while a bright dye such as BB700 is able to fully resolve the CD279-positive cells, leading to more accurate results. Having an additional bright dye for the blue laser expands the choices available for resolving dim populations. This is especially important for instrument configurations with fewer detectors, where fluorochrome options may be limited. For example, CD279 PerCP-Cy5.5 is too dim to be detected on a BD Accuri™ system, while CD279 BB700 provides resolution of the positive and negative populations (Figure 6). With the addition of BB700, there are now more fluorochrome choices for resolving dim markers.

BB700 has less cross-laser excitation on the 405-nm and 561-nm lasers compared to PerCP-Cy5.5, resulting in less spillover into multiple channels, making BB700 more useful for multicolor panels (Table 3). With an excitation max at 485 nm and emission max at 693 nm, BB700 can be excited by the blue laser (488 nm) and detected in the same filter as PerCP-Cy5.5 (for example, 695/40 nm) (Figure 4).

The BB700 portfolio consists of traditional off-the-shelf reagents in multiple sizes as well as BD OptiBuild™ reagents to provide the most options for panel design. BD OptBuild reagents are custom reagents in a convenient 50-µg size that can be ordered the same way as catalog reagents. These custom products are made on demand, and usually ship in less than 72 hours.*

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

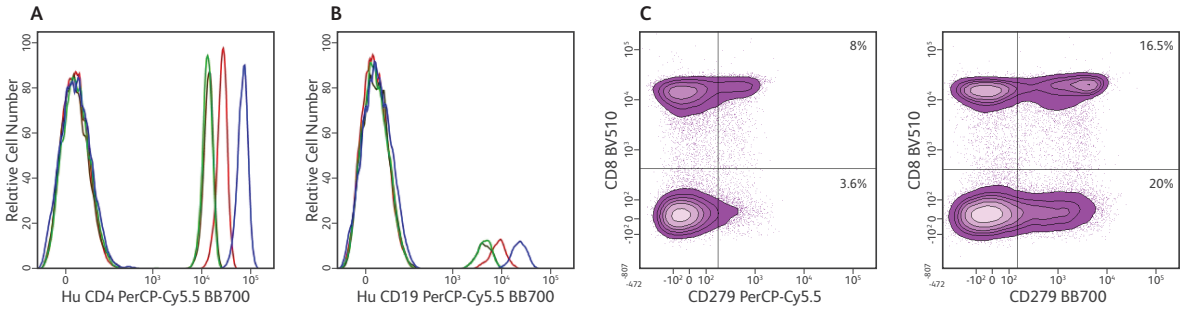

Figure 5. (A) Lysed whole blood stained with Hu CD4 BB700 (clone SK3, BD Biosciences, blue), PerCP-Cy5.5 (clone SK3, BioLegend, green), PerCP-eFluor® 710 (clone SK3, eBioscience, red) or PerCP-Vio®700 (clone REA623, Miltenyi, brown) using the manufacturer's recommended volume per test. **(B)** Lysed whole blood stained with Hu CD19 BB700 (clone SJ25C1, BD Biosciences, blue), PerCP-Cy5.5 (clone SJ25C1, BioLegend, green), PerCP-eFluor® 710 (clone SJ25C1, eBioscience, red) or PerCP-Vio®700 (clone REA675, Miltenyi, brown) using the manufacturer's recommended volume per test. **(C)** Peripheral blood mononuclear cells (PBMCs) were stained with CD279 PerCP-Cy5.5 or BB700 and CD8 BV510. Data shown is gated on CD3-positive cells.

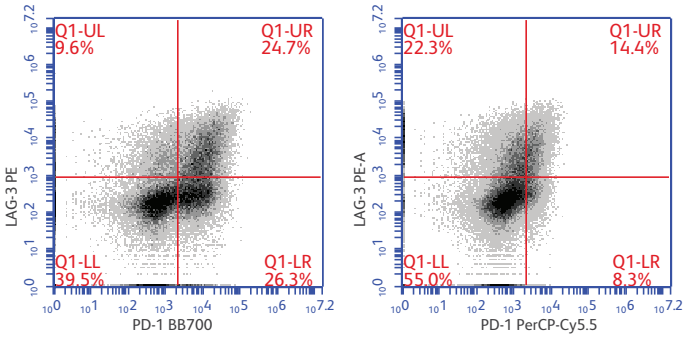

Figure 6. Human PBMCs were stimulated with anti-CD3 and anti-CD28 for 72 hours and stained with PE Mouse Anti-Human LAG-3 and either BB700 (left plot) or PerCP-Cy5.5 (right plot) Mouse Anti-Human PD-1. The cells were also stained with BD Via-Probe™ red nucleic acid stain for live/dead cell discrimination. The two-color dot plots showing correlated expression of PD-1 vs LAG-3 were derived from gated events characteristics of BD Via-Probe red–negative live cells.

	BUV395	BUV496	BUV563	BUV661	BUV737	BUV805	BV421	BV510	BV605	BV650	BV711	BV786
BB700	0%	0%	0%	2%	4%	2%	0%	0%	1%	15%	24%	9%
PerCP-Cy5.5	0%	0%	0%	9%	8%	4%	0%	0%	0%	35%	37%	14%
PerCP-Vio®700	0%	0%	0%	4%	9%	5%	0%	0%	0%	21%	46%	19%
PerCP-eFluor® 710	0%	0%	0%	2%	11%	5%	0%	0%	0%	13%	56%	21%
	FITC	**PE**	**PE-CF594**	**PE-Cy™7**	**APC**	**APC-R700**	**APC-H7**	**PE**	**PE-CF594**	**PE-Cy7**		
BB700	1%	1%	2%	29%	21%	8%	13%	0%	0%	2%		
PerCP-Cy5.5	0%	0%	0%	27%	23%	7%	13%	0%	0%	12%		
PerCP-Vio®700	0%	0%	0%	45%	11%	10%	15%	0%	0%	14%		
PerCP-eFluor® 710	0%	0%	0%	49%	7%	13%	17%	0%	0%	15%		

Table 3. Human CD4 reagents conjugated to various fluorochromes run side by side for a spillover comparison

All data was collected on a BD LSR Fortessa™ X-20 system. To collect data across the most channels, the data from the UV, violet and blue lasers came from one instrument and the data from the red and yellow-green lasers came from another instrument. 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.

Panel design considerations: save bright fluorochromes for the dimmer markers of the panel

Although BB700 PerCP-Cy5.5 and BB515 FITC are detected in the same channel, the differences in brightness should be taken into account when incorporating the fluorochromes into panels. PerCP-Cy5.5 and FITC should be used for high or medium expressed markers in your panel. However, when incorporating BB700 or BB515 into your panel, match the dyes with the dimmer markers in the panel to get the most benefit.

Additionally, the differences in brightness should be taken into account when maximizing population resolution. The resolution for a given antigen (fluorescence parameter) is decreased by the spread due to spillover from other fluorochromes. That is, the addition of a reagent may reduce the resolution of another reagent, potentially affecting overall population resolution and data quality. Spread is most important when considering reagents for co-expressed antigens. It is a not only a function of spillover but also antigen density and fluorochrome brightness. Therefore, on the same antigen, a fluorochrome that has less spillover but is also significantly brighter may actually cause more spread. For example, if comparing BB700 and PerCP-Cy5.5 on a markers of equal antigen density, the spread may be similar or greater with BB700, despite it having less spillover (Figure 7). Figure 7b shows that if the BB700 were titrated down to the brightness of PerCP-Cy5.5 (to mimic switching to a lowly expressed antigen), the reduced spread can be observed. This is also a consideration with BB515, which has less spillover into the PE channel but is significantly brighter than FITC. If using these fluorochromes on highly expressed antigens, keep in mind the potential spread that may be observed due to their brightness. We recommend saving these fluorochromes for the lower expressed antigens of the panel where the brightness will help fully resolve the population and is not likely to affect the resolution of other reagents.

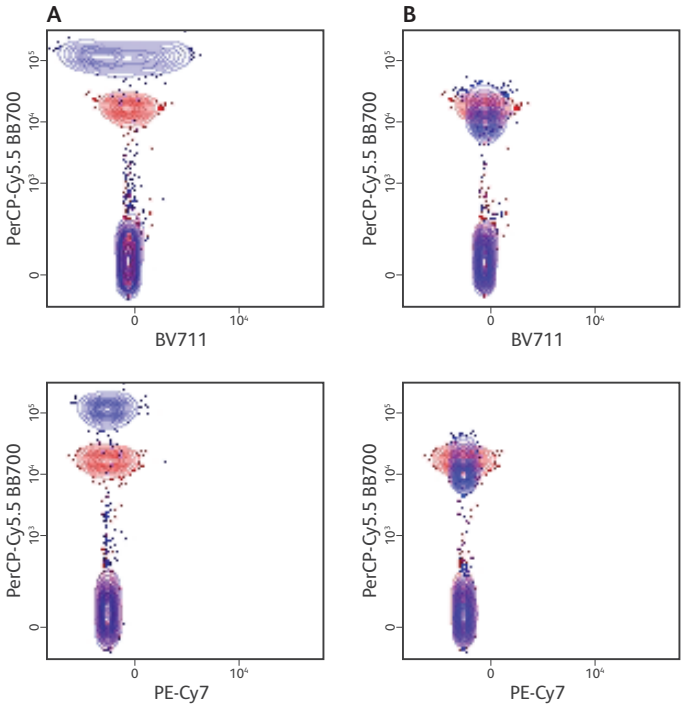

Figure 7. (A) The spread into the BV711 and PE-Cy7 channels from Hu CD4 BB700 (blue) and PerCP-Cy5.5 (red) is shown using the optimal concentration of each reagent. The spread can be seen as the width of the BB700 PerCP-Cy5.5–positive populations. **(B)** Hu CD4 BB700 (blue) was titrated down to a median fluorescence intensity (MFI) similar to the PerCP-Cy5.5 reagent (red). At similar MFI target values, the spread of BB700 into BV711 and PE-Cy7 is similar to or less than that of PerCP-Cy5.5. PE-Cy7 was detected off the 561-nm laser.

Compatible with standard surface and intracellular staining protocols

BD Horizon BB515 and BB700 are compatible with standard buffers used in surface and intracellular staining protocols. When cells are stained prior to the permeabilization step in the presence of strong alcohol based buffers such as BD Phosflow™ perm buffer III, PerCP-Cy5.5 staining is no longer detectable. However, BB700 is compatible with BD Phosflow perm buffer III, making it an ideal choice for intracellular staining conditions (Figure 8).

These reagents also demonstrate compatibility with paraformaldehyde-based fixatives and both EDTA and heparin blood collection tubes.

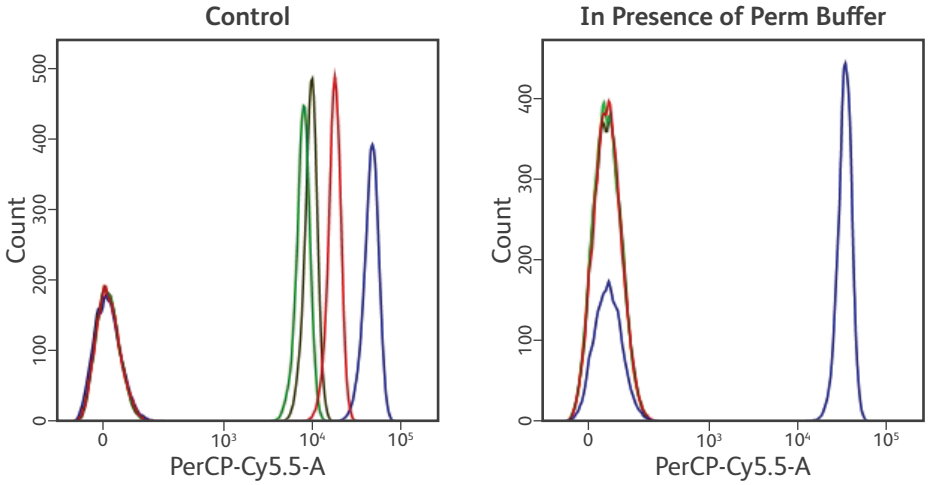

Figure 8. Lysed whole blood was stained with Hu CD4 BB700 (clone SK3, BD Biosciences, blue), PerCP-Cy5.5 (clone SK3, BD Biosciences, green), PerCP-eFluor® 710 (clone SK3, eBioscience, red) or PerCP-Vio®700 (clone REA623, Miltenyi, brown) using the manufacturer's recommended volume per test, washed, incubated with BD Phosflow perm buffer III, washed and run on a cytometer (right). The only reagent that shows staining is CD4 BB700, due to its compatibility with the permeabilization buffer. As a control, lysed whole blood was stained with the same reagents but not incubated with BD Phosflow perm buffer III (left).

Use of BB515 and BB700 in a multicolor panel for the analysis of immune checkpoint expression

T-cell activation is tightly regulated by immune checkpoints, a combination of co-stimulatory and co-inhibitory signals capable of promoting or suppressing T-cell response, respectively. An eight-color panel was designed to assess the expression of co-stimulatory receptors CD137 (4-1BB) and CD134 (OX40), as well as co-inhibitory receptors CD279 (PD-1) and CD366 (TIM-3), within the $CD4^+$ and $CD8^+$ subsets of phytohemagglutinin (PHA)-stimulated $CD3^+$ T cells. The expression of co-signaling receptors is modulated upon T-cell activation, and the level of expression within different subset of cells can be variable. Similarly, the expression of CD3 is known to be significantly down-modulated upon T-cell stimulation. All these factors were taken in consideration when designing the panel. The use of bright dyes (BV421, BB700, APC and PE) allowed for optimal resolution of all the co-signaling receptors in both CD4 and CD8 subsets. As shown in Figure 5c, use of a dim dye such as PerCP-Cy5.5 would underestimate the percentage of cells expressing CD279, so BB700 was chosen instead. In most cases, FITC is an optimal choice for a highly expressed marker such as CD3. However, knowing that CD3 will be down modulated upon PHA stimulation, BB515 was chosen to provide optimal resolution. As a result, up-regulation of all co-stimulatory and co-inhibitory receptors was detected in PHA-stimulated T cells, compared to untreated control (not shown). The multiparameter approach also allowed for the detection of differences in the level of expression of co-signaling receptors within $CD4^+$ and $CD8^+$ subsets. For example, the co-stimulatory receptor CD134 was highly expressed on $CD4^+$ cells, but dimly on $CD8^+$ (Figure 9).

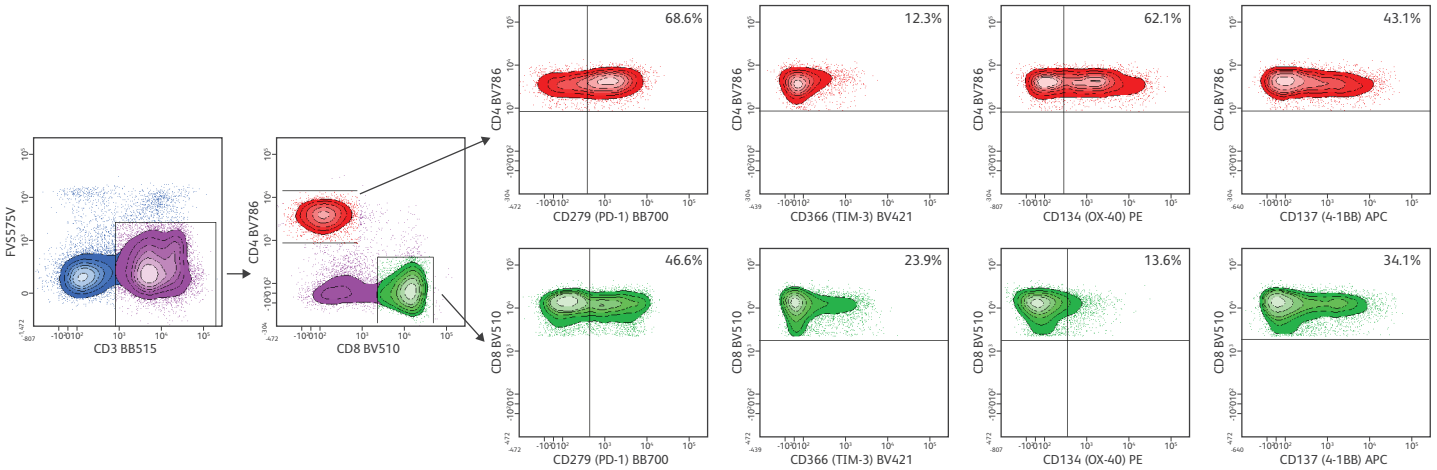

Figure 9. Multicolor panel for the analysis of co-signaling receptor expression

PBMCs were isolated from healthy donors and stimulated with 2% PHA (Sigma) for 12 hours. At the end of the stimulation, cells were collected and stained with a cocktail of antibodies, including CD3 BB515 (Cat. No. 564465), CD4 BV786 (Cat. No. 563877), CD8 BV510 (Cat. No. 563256), CD279 BB700 (Cat. No 566460), CD366 BV421 (Cat. No. 565562), CD134 PE (Cat. No. 555838) and CD137 APC (Cat. No. 550890), in the presence of BD Horizon™ Brilliant Stain Buffer (563794). Cells were then stained with FVS575V viability dye (Cat. No. 565694). Cells were analyzed on a BD FACSCelesta™ system with a Blue/Violet/Red configuration. $CD3^+$ T cells were further divided into $CD4^+$ and $CD8^+$ subsets. Each subset was interrogated for the expression of co-stimulatory and co-inhibitory receptors. While the expression of all the signaling receptors was increased overall compared to unstimulated cells (not shown), a difference in the frequency and level of expression within $CD4^+$ and $CD8^+$ subsets was observed.

A selection of BD Horizon BB515 reagents

Visit **bdbiosciences.com/colors** for a complete list of products.

Description	React	Clone	Size	Cat. no.
CD3	Human	UCHT1	25 Tests	564466
			100 Tests	564465
CD4	Human	RPA-T4	25 Tests	564420
			100 Tests	564419
CD5	Human	UCHT2	25 Tests	564648
			100 Tests	564647
CD7	Human	M-T701	100 Tests	565211
CD11b	Human	ICRF44	25 Tests	564518
			100 Tests	564517
CD11c	Human	B-ly6	25 Tests	564491
			100 Tests	564490
CD19	Human	HIB19	25 Tests	564457
			100 Tests	564456
CD23	Human	M-L233	100 Tests	564555
CD25	Human	2A3	25 Tests	564468
			100 Tests	564467
CD27	Human	M-T271	25 Tests	564643
			100 Tests	564642
CD38	Human	HIT2	25 Tests	564499
			100 Tests	564498
CD45	Human	HI30	25 Tests	564586
			100 Tests	564585
CD45RA	Human	HI100	100 Tests	564552
CD56	Human	B159	25 Tests	564489
			100 Tests	564488
CD80	Human	L307.4	25 Tests	565009
			100 Tests	565008
CD127	Human	HIL-7R-M21	25 Tests	565937
			50 Tests	564423
CD132	Human	TUGh4	25 Tests	566035
			50 Tests	564528
CD279 (PD-1)	Human	EH12.1	25 Tests	565936
			50 Tests	564494
CXCR5 (CD185)	Human	RF8B2	25 Tests	564625
			100 Tests	564624
HLA-DR	Human	G46-6	100 Tests	564516
IgD	Human	IA6-2	25 Tests	565244
			100 Tests	565243
TIM-3 (CD366)	Human	7D3	25 Tests	565569
			100 Tests	565568

Description	React	Clone	Size	Cat. no.
CD5	Mouse	53-7.3	50 µg	565504
CD8a	Mouse	53-6.7	25 µg	564459
			0.1 mg	564422
CD11b	Mouse	M1/70	25 µg	564455
			0.1 mg	564454
CD19	Mouse	1D3	25 µg	564531
			0.1 mg	564509
CD23	Mouse	B3B4	50 µg	564637
CD25	Mouse	PC61	25 µg	564458
			0.1 mg	564424
CD43	Mouse	S7	50 µg	564646
CD62L	Mouse	MEL-14	0.1 mg	565261
CD105	Mouse	MJ7/18	25 µg	565944
			50 µg	564744
CD117	Mouse	2B8	0.1 mg	564481
CD138	Mouse	281-2	25 µg	566207
			50 µg	564511
CD223	Mouse	C9B7W	25 µg	566210
			50 µg	564672
CD278	Mouse	7E.17G9	25 µg	566214
			50 µg	564592
CD370 (Clec9A)	Mouse	10B4	25 µg	566033
			50 µg	565320
I-A/I-E	Mouse	2G9	0.1 mg	565254
IL-23R	Mouse	O78-1208	25 µg	565011
			50 µg	566212
Ly-6A/E	Mouse	D7	50 µg	565397
Siglec-F	Mouse	E50-2440	25 µg	566211
			50 µg	564514

A selection of BD Horizon BB700 reagents.

Visit **bdbiosciences.com/colors** for a complete list of products.

Description	React	Clone	Size	Cat. no.
CD4	Human	SK3	25 Tests	566393
			100 Tests	566392
CD14	Human	MϕP9	25 Tests	566466
			100 Tests	566465
CD19	Human	SJ25C1	25 Tests	566397
			100 Tests	566396
CD56	Human	B159	25 Tests	566401
			100 Tests	566400
CD127	Human	HIL-7R-M21	25 Tests	566399
			100 Tests	566398
CD279 (PD-1)	Human	EH12.1	25 Tests	566461
			100 Tests	566460
IFN-γ	Human	B27	25 Tests	566395
			100 Tests	566394
IL-2	Human	MQ1-17H12	25 Tests	566406
			100 Tests	566405

Description	React	Clone	Size	Cat. no.
CD4	Mouse	RM4-5	25 µg	566408
			0.1 mg	566407
CD8α	Mouse	53-6.7	25 µg	566410
			0.1 mg	566409
CD11b	Mouse	M1/70	25 µg	566417
			0.1 mg	566416
CD19	Mouse	1D3	25 µg	566412
			0.1 mg	566411
CD117	Mouse	2B8	25 µg	566415
			0.1 mg	566414

A selection of BD OptiBuild BB700 reagents

Description	React	Clone	Size	Cat. no.
CD3	Human	HIT3a	50 µg	742207
CD8	Human	HIT8a	50 µg	742229
CD11b	Human	ICRF44	50 µg	742210
CD16	Human	B73.1	50 µg	742286
CD22	Human	HIB22	50 µg	742214
CD32	Human	FLI8.26	50 µg	742216
CD33	Human	WM53	50 µg	742217
CD34	Human	563	50 µg	742246
CD42b	Human	HIP1	50 µg	742219
CD45RA	Human	5H9	50 µg	742249
CD49e	Human	IIA1	50 µg	742228
CD54	Human	HA58	50 µg	742221
CD66	Human	B1.1/CD66	50 µg	742248
CD117	Human	104D2	50 µg	742284
CD141	Human	1A4	50 µg	742245
CD206	Human	19.2	50 µg	742237
CD325	Human	8C11	50 µg	742273
HLA-A2	Human	BB7.2	50 µg	742247
HLA-ABC	Human	G46-2.6	50 µg	742223
HLA-DR, DP, DQ	Human	Tu39	50 µg	742224
IgG	Human	G18-145	50 µg	742235

Description	React	Clone	Size	Cat. no.
CD1d	Mouse	1B1	50 µg	742147
CD3e	Mouse	500A2	50 µg	742099
CD4	Mouse	H129.19	50 µg	742116
CD5	Mouse	53-7.3	50 µg	742083
CD8b	Mouse	H35-17.2	50 µg	742198
CD11a	Mouse	2D7	50 µg	742092
CD27	Mouse	LG.3A10	50 µg	742135
CD31	Mouse	390	50 µg	742123
CD38	Mouse	90/CD38	50 µg	742132
CD40	Mouse	3/23	50 µg	742136
CD45RA	Mouse	14.8	50 µg	742113
CD49b	Mouse	HMα2	50 µg	742140
CD54	Mouse	3E2	50 µg	742100
CD86	Mouse	GL1	50 µg	742120
CD90.2	Mouse	30-H12	50 µg	742082
CD138	Mouse	281-2	50 µg	742124
CD184	Mouse	2B11/CXCR4	50 µg	742171
CD193	Mouse	83103	50 µg	742079
CXCR5	Mouse	2G8	50 µg	742173
CD357 (GITR)	Mouse	DTA-1	50 µg	742262
KLRG1	Mouse	2F1	50 µg	742199

*US shipping time is typically overnight. Shipping times vary by region according to shipping schedules

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.

23-19554-00

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

bdbiosciences.com