BD Horizon Brilliant™ Ultraviolet Reagents

Features

- UV (355-nm)–excitable dyes
- Provide great population resolution
- Low spillover into most detectors
- More choice and flexibility for multicolor panel design

BD Horizon Brilliant™ ultraviolet (BUV) polymer dyes are UV-excitable dyes that have been developed exclusively by BD Life Sciences to expand the multicolor capabilities of flow cytometers equipped with a 355-nm laser. Currently available UV-excitable fluorochromes are so dim that they are not practical for immunophenotyping applications. However, BD Horizon™ BUV dyes provide great population resolution, even for dim markers. Additionally, these dyes allow markers to be spread over more lasers, reducing the compensation requirements of the panel. BD Horizon BUV reagents allow researchers to get the most from instruments equipped with a 355-nm laser. The reagents are not recommended for instruments equipped with a 375-nm laser.

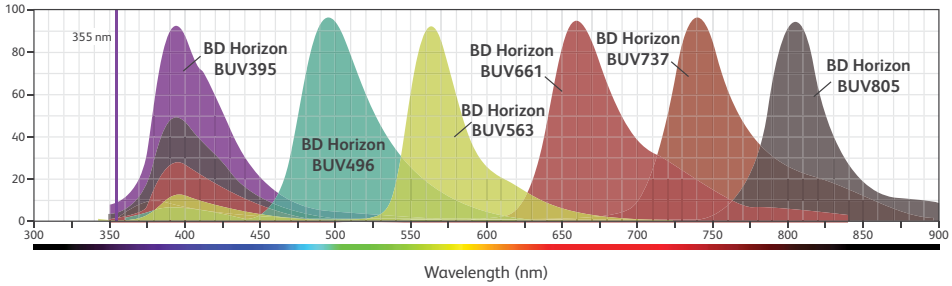


Figure 1. Excitation and emission profiles of BD Horizon BUV395, BUV496, BUV563, BUV661, BUV737 and BUV805.

BD Horizon™ BUV395

BD Horizon Brilliant™ Ultraviolet 395 (BUV395) is a base polymer dye and is optimal for multicolor flow cytometry because it has virtually no spillover into any other detector (Table 3). Additionally, other fluorochromes have little to no spillover into the BUV395 detector. BUV395 allows an additional color to be added to a panel without increasing the complexity of compensation requirements.

BUV395 clearly resolves both dim and abundant populations. In many cases, BUV395 reagents will have brightness similar to or greater than FITC reagents.

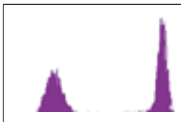
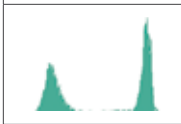
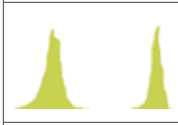
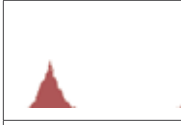
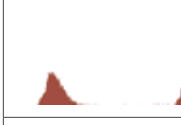
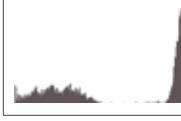
BD Horizon Brilliant ultraviolet tandem dyes

BUV496, BUV563, BUV661, BUV737, and BUV805 are tandem dyes consisting of BUV395 and an acceptor dye with emission indicated by the name of the dye. Due to the excitation of the acceptor dye by other laser lines, there may be spillover into other detectors (Table 3). The tandem dyes can be chosen based on brightness requirements, spillover into other dyes in the panel, and reagent availability. For example, if a bright dye is needed, BUV737, BUV563 or BUV661 may be optimal choices (Table 1). If spillover into other detectors is the main concern, BUV496 or BUV805 may provide optimal results. If using three BUV dyes, BUV395, BUV496 and BUV737 is a good place to start. Depending on the needs of the panel, BUV661, BUV805 or BUV563 may be substituted for BUV496 or BUV737 to provide more optimal results.

More choice and flexibility for multicolor panel design

The family of BD Horizon Brilliant ultraviolet reagents provides more choice for multicolor flow cytometry, making multicolor panel design easier. Using these dyes with other fluorochromes offered by BD Life Sciences enables detection of more than 18 fluorescence parameters from a single sample.

Managing spillover between reagents can be one of the more difficult elements of multicolor panel design. By spreading markers over multiple lasers, the overall compensation requirements of a panel can be reduced. For example, by assigning one marker to each laser, a 5-color panel with minimal compensation requirements can be run on an instrument equipped with UV, violet, blue, red, and yellow-green lasers (Table 2). The availability of UV-excitable reagents makes it easier to design panels with less spillover. This alleviates one of the most difficult elements of multicolor panel design.

	Fluorochrome	Filter	Stain index	Relative resolution
	BUV395	379/28 BP	63	
	BUV496	515/30 BP, 450LP	59	
	BUV563	585/15, 535LP	240	
	BUV661	670/25 BP, 630LP	321	
	BUV737	740/35 BP, 690LP	196	
	BUV805	820/60 BP, 770LP	44	

Relative brightness key: Dim Moderate Bright Brightest

Table 1. Lysed whole blood stained with various human CD4 reagents
Data shown is on lymphocytes. Relative stain index is dependent on instrument configuration including lasers, filters, and laser power.

Laser	Filter	Fluorochrome	Human T-cell panel	Human B-cell panel	Mouse B-cell panel
Blue 488 nm	530/30	FITC	CD8	IgD	IgD
Yellow-Green 561 nm	610/20	PE-CF594	CD27	CD38	IgM
Red 640 nm	670/30	APC	CD45RA	IgM	CD21
Violet 405 nm	450/40	BV421	CD3	CD27	CD23
Ultraviolet 355 nm	379/28	BUV395	CD4	CD19	CD19

Table 2. Example of instrument configuration and possible panels to achieve a 5-color panel with minimal compensation requirements

Laser		Spillover into other detectors					
UV		BUV395	BUV496	BUV563	BUV661	BUV737	BUV805
	BUV395		12%	0%	0%	0%	0%
	BUV496	4%		16%	5%	1%	0%
	BUV563	2%	1%		16%	3%	1%
	BUV661	1%	0%	0%		30%	11%
	BUV737	2%	0%	0%	1%		52%
	BUV805	1%	0%	0%	0%	0%	
Violet		BV421	BV510	BV605	BV650	BV711	BV786
	BUV395	0%	0%	0%	0%	0%	0%
	BUV496	1%	27%	5%	1%	0%	0%
	BUV563	0%	1%	5%	1%	0%	0%
	BUV661	0%	0%	0%	8%	2%	0%
	BUV737	0%	0%	0%	0%	4%	3%
	BUV805	0%	0%	0%	0%	0%	1%
Blue		FITC	PE	PE-CF594	PE-Cy™5	PerCP-Cy™5.5	PE-Cy™7
	BUV395	0%	0%	0%	0%	0%	0%
	BUV496	4%	1%	0%	0%	0%	0%
	BUV563	3%	40%*	26%*	1%*	2%	1%*
	BUV661	0%	0%	0%	0%	0%	0%
	BUV737	0%	0%	0%	0%	2%	9%
	BUV805	0%	0%	0%	0%	0%	0%
Red					APC	Alexa Fluor® 700	APC-Cy7
	BUV395				0%	0%	0%
	BUV496				0%	0%	0%
	BUV563				0%	0%	0%
	BUV661				42%	19%	3%
	BUV737				1%	45%	11%
	BUV805				0%	0%	1%

Table 3. BD Horizon Brilliant ultraviolet dye spillover into channels on each laser line
This table shows relative spillover values of the dyes, since spillover values obtained can vary depending on the filter used and PMT voltage. Yellow and red fill colors denote where there is more spillover between dyes. The green fill color denotes a small degree of spillover between dyes.
** This data was collected on an instrument configured to detect PE and its subsequent tandems off the Yellow-Green laser.*

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