BD Biosciences Fluorochrome Reference Chart

Visit **bdbiosciences.com/colors** for detailed information about our newest fluorochromes and instrumentation.

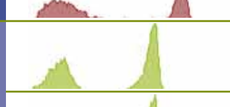
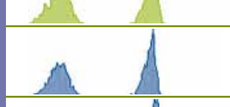
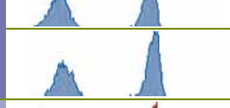
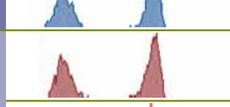
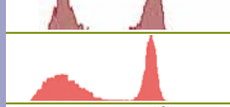
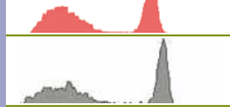
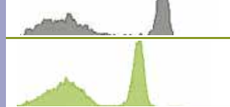
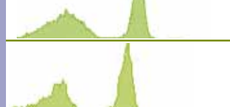
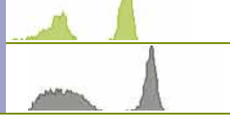
To select your optimal combination of fluorochromes, visit **bdbiosciences.com/spectra** to use an interactive fluorescence spectrum tool.

23-9582-08

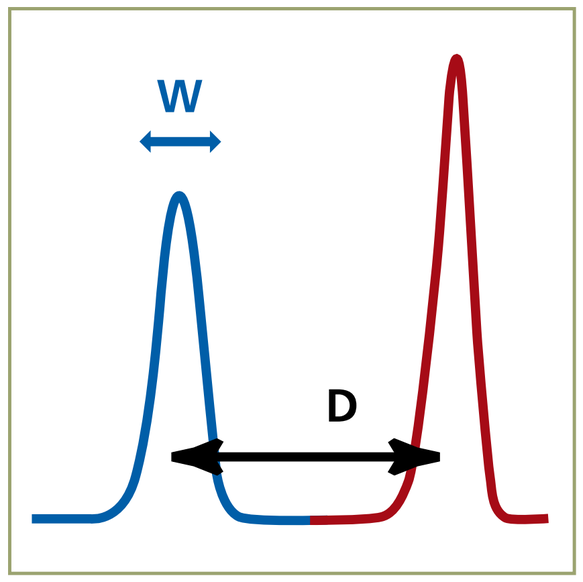
Instrument	Excitation Laser Line (nm)	Fluorescence Channel	Fluorochromes provided by BD Biosciences			
BD Accuri™ C6	488	FL1 Green	FITC	Alexa Fluor® 488		
		FL2 Yellow	PE	PI		
		FL3 Red	7-AAD	PerCP	PerCP-Cy™5.5	PE-Cy™7
	640	FL4 Red	APC	Alexa Fluor® 647		
BD FACSCalibur™	488	FL1 Green	FITC	Alexa Fluor® 488		
		FL2 Yellow	PE	PI		
		FL3 Red	7-AAD	PE-Cy™5	PerCP	PerCP-Cy5.5
	635	FL4 Red	APC	Alexa Fluor® 647		PE-Cy7
BD FACSVerse™*	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon™ PE-CF594[a]	PE-Texas Red®[a]		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	640[a]	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700[a]			
		Infrared	BD APC-H7	APC-Cy7		
	405[a]	Blue	Brilliant Violet™ 421	BD Horizon™ V450	VPD450	Pacific Blue™
		Green	BD Horizon™ V500	AmCyan		
BD FACSCanto™ II	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594[a]	PE-Texas Red®[a]		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	561[b]	Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	PE-Cy5			
		Infrared	PE-Cy7			
	633	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700[a]			
		Infrared	BD APC-H7	APC-Cy7		
	405[a]	Blue	Brilliant Violet™ 421	BD Horizon™ V450	VPD450	Pacific Blue™
		Green	BD Horizon V500	BD Horizon V500-C	AmCyan	
BD LSRFortessa™ and Special Order BD LSRFortessa (typical setup)[b]	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	532[b] or 561[b]	Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	PE-Cy5			
		Infrared	PE-Cy7			
	640	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700			
		Infrared	BD APC-H7	APC-Cy7		
	405	Blue	Brilliant Violet™ 421	BD Horizon™ V450	VPD450	Pacific Blue™
		Green	BD Horizon V500	AmCyan		
		Orange	Brilliant Violet™ 605[a]			
	355	Blue	Hoechst 33342			
BD FACSAria™ III and Special Order BD FACSAria (typical setup)[b]	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	561	Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	PE-Cy5			
		Infrared	PE-Cy7			
	640	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700			
		Infrared	BD APC-H7	APC-Cy7		
	405	Blue	Brilliant Violet™ 421	BD Horizon™ V450	VPD450	Pacific Blue™
		Green	BD Horizon V500	AmCyan		
		Orange	Brilliant Violet™ 605[a]			
	375[b]	Blue	Hoechst 33342			
BD Influx™	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	532 or 561	Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	PE-Cy5			
		Infrared	PE-Cy7			
	640	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor®700			
		Infrared	BD APC-H7	APC-Cy7		
	405	Blue	Brilliant Violet™ 421	BD Horizon™ V450	VPD450	Pacific Blue™
		Green	BD Horizon V500	AmCyan		
		Orange	Brilliant Violet™ 605[a]			
	375	Blue	Hoechst 33342			
BD FACSJazz™	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE			
		Red	PerCP-Cy5.5			
		Infrared	PE-Cy7			
	640[a]	Red	APC	Alexa Fluor® 647		
		Infrared	BD APC-H7	APC-Cy7		
	405[a]	Blue	Brilliant Violet™ 421	BD Horizon™ V450	Pacific Blue™	
		Green	BD Horizon V500			

[a]*Available through laser and/or detector options.*
[b]*More laser and detector options are available through the Special Order Research Products (SORP) program.*

Brightness of various fluorochrome conjugates

Relative Brightness		Reagent	Filter
BRIGHTEST		Brilliant Violet™ 421	450/50
		PE	575/26
		Brilliant Violet 605	610/20
		BD Horizon PE-CF594	610/20
		PE-Cy5	670/14
		APC	660/20
BRIGHT		PE-Cy7	780/60
		Alexa Fluor® 647	660/20
		PerCP-Cy5.5	695/40
MODERATE		Alexa Fluor® 488	530/30
		FITC	530/30
		BD Horizon V450	450/50
		Pacific Blue™	450/50
DIM		Alexa Fluor® 700	730/45
		PerCP	695/40
		APC-Cy7	780/60
		AmCyan	525/20
		BD Horizon V500	525/20
		BD APC-H7	780/60

Freshly isolated lymphocytes, stained with anti-human CD4 (RPA-T4) conjugated with various fluorochromes run on a BD LSR II flow cytometer. The fluorochromes were ranked based on observed stain index values. This chart is meant as a guideline of relative stain indices of various fluorochromes. Observed relative stain indices may vary depending on instrument, instrument configuration, reagents, and cell type used.



Stain Index = D/W

Resolution sensitivity (the ability to resolve a dim positive signal from background) is a function of the difference between positive and background peak means (D) and the spread of the background peak (W). The stain index is a metric that captures both of these factors.

Choose a winning combination - Guidelines for selecting reagents for multicolor flow cytometry

1 The basics: Know your instrument
Reagent selection starts with your instrument configuration. The lasers and detectors in your configuration dictate how well your cytometer can excite and measure a given fluorochrome, and whether you have enough detectors to read out a given combination of fluorochromes.

2 Fluorochromes: Go for the bright
Rank available dyes according to their intrinsic brightness on a particular instrument (when configured with a specified set of lasers and filters).

3 Minimize spillover
As soon as cells are stained with multiple reagents, spectral overlap (or spillover) becomes an issue. The more colors you attempt to resolve on any particular cell, the more spillover impacts sensitivity. We use compensation, an adjustment applied to all colors, to correct for spillover. For example, a cell population fluorescing only in FITC will show no PE fluorescence, on average, but will likely exhibit more spread in the PE detector after compensation than completely unstained cells.

4 Colors and specificities: Define winning combinations
Once the fluorochromes to be used have been defined, you can begin to match antibody specificities to particular fluorochromes. Generally, reserve the brightest fluorochromes for dim antigens, and vice versa, but avoid spillover from bright cell populations into detectors requiring high sensitivity for those populations.

5 Tandem dyes
APC-Cy7, and to a lesser extent, PE-Cy7, can degrade in the presence of light, fixative, and elevated temperatures so that they emit in the parent dye detector (APC or PE). By minimizing the exposure of samples to light, heat, and formaldehyde-based fixatives, this problem can be largely avoided. For more stable tandem dyes, BD now offers BD APC-H7 conjugated antibodies.

6 Validation
Use controls (such as fluorescence-minus-one, or FMO) to validate your selected multicolor reagent cocktail. FMO controls help define the contribution of spillover to the background in a given detector, and are therefore useful in gauging the sensitivity of that detector in the context of a certain reagent cocktail.

*For additional guidelines, visit **bdbiosciences.com/colors** to download the Application Note "Selecting Reagents for Multicolor Flow Cytometry."*

* Capable of detecting 8 colors simultaneously (4 blue laser, 2 red laser, 2 violet laser)