BD Biosciences Fluorochrome Reference Chart

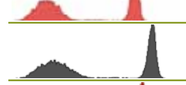
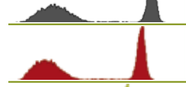
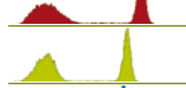
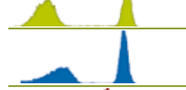
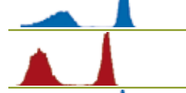
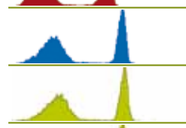
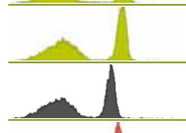
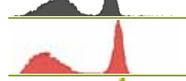
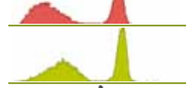
Visit **bdbiosciences.com/colors** for detailed information about our newest fluorochromes and instrumentation.

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

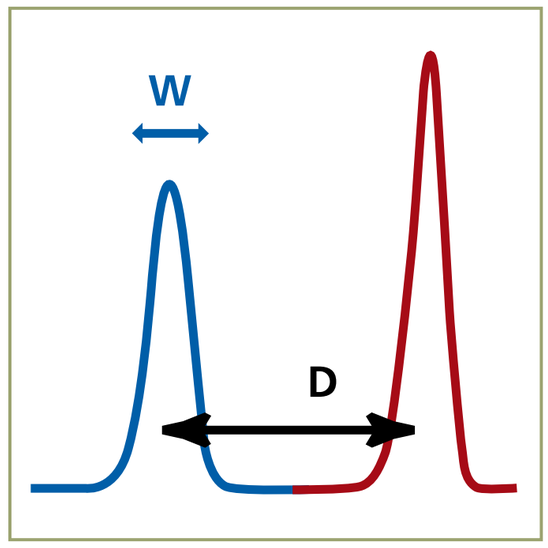
Instrument	Excitation Laser Line (nm)	Fluorescence Channel	Fluorochromes provided by BD Biosciences				
Accuri® C6	488	FL1 Green	FITC	Alexa Fluor® 488			
		FL2 Yellow	PE	PI			
		FL3 Red	7-AAD	PerCP	PerCP-Cy™5.5	PE-Cy™7	
	640	FL4 Red	APC	Alexa Fluor® 647			
BD FACSCalibur™	488	FL1 Green	FITC	Alexa Fluor®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

[a]*APC and PE-Cy5 may be used together on instruments with cross-beam compensation.* [b]*Available through laser and/or detector options.*
[c]*More laser and detector options are available through the Special Order Research Products (SORP) program.*

Stain index of various fluorochrome conjugates on a BD™ LSR II

	Reagent	Clone	Filter	Stain Index
	PE	RPA-T4	575/26	305
	APC	RPA-T4	660/20	263
	PE-Cy5	RPA-T4	695/40	198
	Alexa Fluor® 647	RPA-T4	660/20	184
	PE-Cy7	RPA-T4	780/60	122
	PerCP-Cy5.5	RPA-T4	695/40	99
	Alexa Fluor® 488	RPA-T4	530/30	68
	BD Horizon V450	RPA-T4	450/50	65
	Alexa Fluor® 700	RPA-T4	720/40	64
	Pacific Blue™	RPA-T4	450/50	63
	FITC	RPA-T4	530/30	43
	AmCyan	RPA-T4	525/50	37
	APC-Cy7	RPA-T4	780/60	36
	PerCP	RPA-T4	695/40	30
	BD Horizon V500	RPA-T4	525/50	27
	BD APC-H7	RPA-T4	780/60	25

Freshly isolated lymphocytes, stained with anti-human CD4 antibodies conjugated with various fluorochromes run on a BD LSR II flow cytometer. This chart is meant as a guideline of relative stain indices of various fluorochromes. Observed relative stain indices may vary depending on instrument configurations and reagents used.



Stain Index = D/W

Resolution sensitivity (the ability to resolve a dim positive signal from background) depends upon 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.

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

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."*

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