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	Laser	Excitation Laser Line (nm)	Fluorescence Channel	Fluorochromes provided by BD Biosciences			
BD FACSArray™ bioanalyzer	Green Diode	532	Yellow	PE			
			Far Red	PerCP-Cy5.5	PE-Cy7		
	Red Diode	635	Red	APC	Alexa Fluor® 647		
			Infrared	BD APC-H7	APC-Cy7		
***BD FACSCalibur™ flow cytometry system**	Argon	488	FL1 Green	FITC	Alexa Fluor® 488		
			FL2 Yellow	PE			
			FL3 Red	PE-Cy5[a]	PerCP	PerCP-Cy5.5	PE-Cy7
	Red Diode	635	FL4 Red	APC[a]	Alexa Fluor® 647		
***BD FACSCanto™ flow cytometry system**	Solid State	488	Green	FITC	Alexa Fluor® 488		
			Yellow	PE			
			Red	PerCP	PerCP-Cy5.5		
			Infrared	PE-Cy7			
	HeNe	633	Red	APC	Alexa Fluor® 647		
			Infrared	BD APC-H7	APC-Cy7		
***†BD FACSCanto™ II flow cytometry system**	Solid State	488	Green	FITC	Alexa Fluor® 488		
			Yellow	PE			
			Orange	PE-Texas Red®[b]			
			Red	PerCP	PerCP-Cy5.5		
			Infrared	PE-Cy7			
	HeNe	633	Red	APC	Alexa Fluor® 647		
			Far Red	Alexa Fluor® 700[b]			
			Infrared	BD APC-H7	APC-Cy7		
	Solid State[b]	405	Green	BD Horizon™ 500[b]	AmCyan[b]		
			Blue	BD Horizon™ V450[b]	Pacific Blue™,[b]		
Preconfigured BD™ LSR II (typical setup)[d]	Solid State	488	Green	FITC	Alexa Fluor® 488		
			Yellow	PE			
			Orange	PE-Texas Red®			
			Red	PerCP	PE-Cy5[a]	PerCP-Cy5.5	
			Infrared	PE-Cy7			
	Solid State	640	Red	APC[a]	Alexa Fluor® 647		
			Far Red	Alexa Fluor® 700			
			Infrared	BD APC-H7	APC-Cy7		
	Solid State	405	Green	BD Horizon V500	AmCyan		
			Blue	BD Horizon V450	Pacific Blue™		
Special Order BD™ LSR II **Special Order BD LSRFortessa™** (typical setup)[d]	Solid State	488	Green	FITC	Alexa Fluor® 488		
			Yellow	PE			
			Orange	PE-Texas Red®			
			Red	PerCP	PE-Cy5[a]	PerCP-Cy5.5	
			Infrared	PE-Cy7			
	Solid State	532 or 561	Yellow	PE			
			Orange	PE-Texas Red®			
			Red	PE-Cy5[a]			
			Infrared	PE-Cy7			
	Solid State	640	Red	APC[a]	Alexa Fluor® 647		
			Far Red	Alexa Fluor® 700			
			Infrared	BD APC-H7	APC-Cy7		
	Solid State	405	Green	BD Horizon V500	AmCyan		
			Blue	BD Horizon V450	Pacific Blue™		
BD FACSAria™ cell sorter family[c] (typical setup)[d]	Solid State	488	Green	FITC	Alexa Fluor® 488		
			Yellow	PE			
			Orange	PE-Texas Red®			
			Red	PerCP	PE-Cy5[a]	PerCP-Cy5.5	
			Infrared	PE-Cy7			
	Solid State[b]	561	Yellow	PE			
			Orange	PE-Texas Red®			
			Red	PE-Cy5[a]			
			Infrared	PE-Cy7			
	HeNe	640	Red	APC[a]	Alexa Fluor® 647		
			Far Red	Alexa Fluor® 700			
			Infrared	BD APC-H7	APC-Cy7		
	Solid State[b]	405	Green	BD Horizon V500	AmCyan		
			Blue	BD Horizon V450	Pacific Blue™		
BD Influx™ cell sorter	Solid State	488	Green	FITC	Alexa Fluor® 488		
			Yellow	PE			
			Orange	PE-Texas Red®			
			Red	PE-Cy5	PerCP-Cy5.5		
			Infrared	PE-Cy7			
	Solid State	532 or 561	Yellow	PE			
			Orange	PE-Texas Red®			
			Red	PE-Cy5			
			Infrared	PE-Cy7			
	Solid State	640	Red	APC	Alexa Fluor® 647		
			Far Red	Alexa Fluor®700			
			Infrared	BD APC-H7	APC-Cy7		
	Solid State	405	Green	BD Horizon V500	AmCyan		
			Blue	BD Horizon V450	Pacific Blue™		

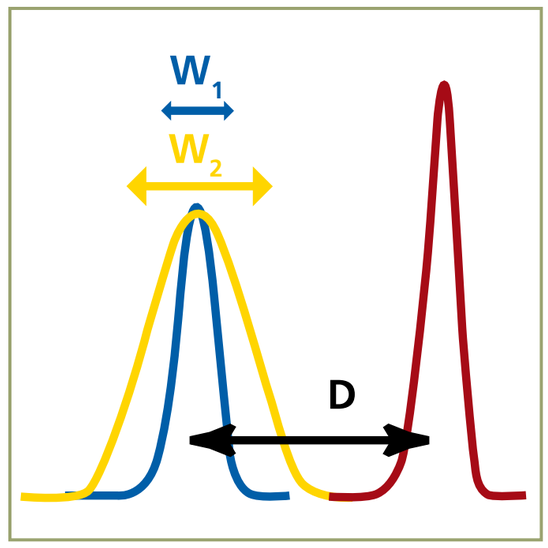
[a]APC and PE-Cy5 may be used together on instruments with cross-beam compensation. [b]Available through laser and/or detector options. [c]BD FACSAria™ and BD FACSAria™ II [d]More laser and detector options are available through the Special Order Research Products (SORP) program.

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

Reagent	Clone	Filter	Stain Index
PE	RPA-T4	575/26	305
APC[1]	RPA-T4	660/20	263
PE-Cy™5[2]	RPA-T4	695/40	198
Alexa Fluor® 647[1]	RPA-T4	660/20	184
PE-Cy™7	RPA-T4	780/60	122
PerCP-Cy™5.5[2]	RPA-T4	695/40	99
Alexa Fluor® 488[3]	RPA-T4	530/30	68
BD Horizon™ V450[5]	RPA-T4	450/50	65
Alexa Fluor® 700	RPA-T4	720/40	64
Pacific Blue™,[5]	RPA-T4	450/50	63
FITC[3]	RPA-T4	530/30	43
AmCyan[6]	RPA-T4	525/50	37
APC-Cy7[4]	RPA-T4	780/60	36
PerCP[2]	RPA-T4	695/40	30
BD Horizon™ V500[6]	RPA-T4	525/50	27
BD APC-H7[4]	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.

[1, 2, 3, 4, 5, 6] Fluorochromes listed with the same superscript number are read in the same detector, and thus would not normally be used in combination.



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). W_1 and W_2 represent background peaks with different spreads. 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.

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

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 In Vitro Diagnostic Use.

† Seven- and eight-color assays on this device are for Research Use Only.

Unless otherwise specified, all products are for Research Use Only.

Class I (1) laser product

APC-Cy7: US patent 5,714,386

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