

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.

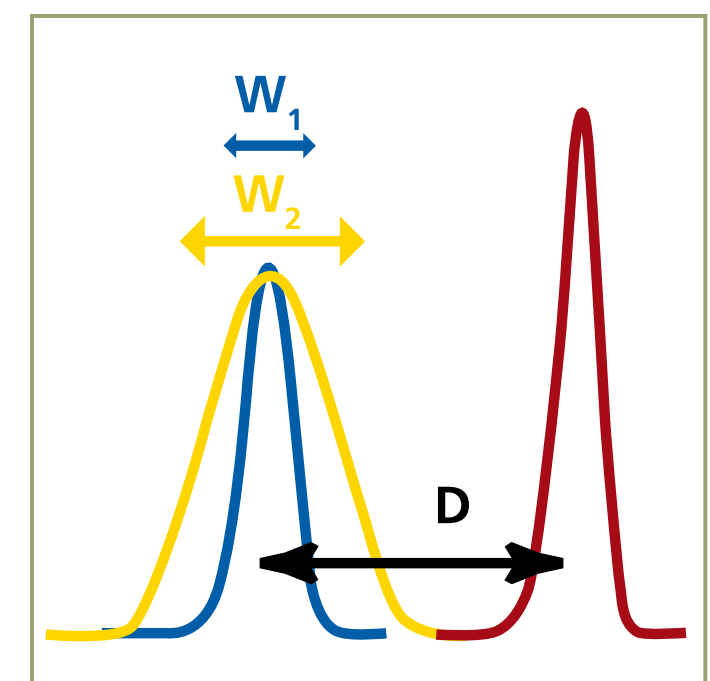
Instrument	Laser	Excitation Laser Line (nm)	Fluorescence Channel	Fluorochromes provided by BD Biosciences
BD FACSAria™ bioanalyzer	Green Diode	532	Yellow	PE
		635	Far Red	PerCP-Cy5.5 PE-Cy7
	Red Diode		Red	APC Alexa Fluor® 647
*BD FACSCalibur™ flow cytometry system	Argon	488	FL1 Green	FITC Alexa Fluor®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

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-Cy5 ²	RPA-T4	695/40	198
Alexa Fluor® 647 ¹	RPA-T4	660/20	184
PE-Cy7 ³	RPA-T4	780/60	122
PerCP-Cy5.5 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). W₁ and W₂ represent background peaks with different spreads. The stain index is a metric that captures both of these factors.

* 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

Cy™ is a trademark of Amersham Biosciences Corp.

Pacific Blue is a trademark and Alexa Fluor and Texas Red are registered trademarks of Molecular Probes, Inc. Alexa Fluor®, Texas Red®, and Pacific Blue™ dye antibody conjugates are sold under license from Molecular Probes, Inc., for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430) and Pacific Blue™ dye are covered by pending and issued patents.

BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company.

© 2009 BD

23-9582-04

^aAPC and PE-Cy5 may be used together on instruments with cross-beam compensation. ^bAvailable through laser and/or detector options. ^cBD FACSAria™ and BD FACSAria™ II ^dMore laser and detector options are available through the Special Order Research Products (SORP) program.

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."

