

# BD Biosciences Fluorochrome Reference Chart

## Typical Instrument Configuration

Instrument	Laser	Excitation Laser Line (nm)	Fluorescence Channel	BD Biosciences Fluorochromes				
BD FACScan™	Argon (L1)	488	FL1 Green	FITC	Alexa Fluor® 488			
			FL2 Yellow	PE				
			FL3 Red	PE-Texas Red®	PE-Cy5*	PerCP	PerCP-Cy5.5	PE-Cy7
BD FACSCalibur™	Argon (L1)	488	FL1 Green	FITC	Alexa Fluor® 488			
	Red Diode (L2)	635	FL2 Yellow	PE				
			FL3 Red	PE-Texas Red®	PE-Cy5*	PerCP	PerCP-Cy5.5	PE-Cy7
BD FACStar™ Plus (typical setup)	Argon (L1)	488	FL1 Green	FITC	Alexa Fluor® 488			
	HeNe (L2)	633	FL2 Yellow	PE				
			FL3 (1) Red	PE-Texas Red®	PE-Cy5*	PerCP-Cy5.5	PE-Cy7	
			FL3 (2) Red	APC*	Alexa Fluor® 647			
			FL4 InfraRed	APC-Cy7				
BD FACSVantage™ SE (typical setup)	Argon (L1)	488	FL1 Green	FITC	Alexa Fluor® 488			
	Krypton (L2) HeNe (L2 or L3)	407 633	FL2 Yellow	PE				
			FL6 Red	PE-Texas Red®	PE-Cy5*	PerCP-Cy5.5		
			FL3 InfraRed	PE-Cy7				
			FL4 (1) Blue	Alexa Fluor® 405	Pacific Blue®			
BD™ LSR (typical setup)	Argon (L1)	488	FL1 Green	FITC	Alexa Fluor® 488			
	HeCd (L2) HeNe (L3)	325 633	FL2 Yellow	PE				
			FL3 Red	PE-Texas Red®	PE-Cy5*	PerCP	PerCP-Cy5.5	PE-Cy7
			FL4, FL5 Blue					
			FL6 Red	APC*	Alexa Fluor® 647	APC-Cy7		
BD™ LSR II (typical setup)	Argon (L1)	488	Green	FITC	Alexa Fluor® 488			
	HeNe (L2)	633	Yellow	PE				
			Red	PE-Texas Red®	PE-Cy5*	PerCP	PerCP-Cy5.5	
			Infra Red	PE-Cy7				
	UV (L3)	355	Red	APC*	Alexa Fluor® 647			
			Infra Red	APC-Cy7				
	Violet (L4)	405	Blue	Alexa Fluor® 405	Pacific Blue®			
BD FACSAria™ (typical setup)	Argon (L1)	488	Green	FITC	Alexa Fluor® 488			
	HeNe (L2)	633	Yellow	PE				
			Red	PE-Texas Red®	PE-Cy5*	PerCP		
			Far Red	PerCP-Cy5.5				
			InfraRed	PE-Cy7				
Violet (L3)	407	Red	APC*	Alexa Fluor® 647				
		InfraRed	APC-Cy7					
		Blue	Alexa Fluor® 405	Pacific Blue®				
BD FACSTArray™ (typical setup)	Green Diode (L1)	532	Yellow	PE				
	Red Diode (L2)	635	Far Red	PerCP-Cy5.5	PE-Cy7			
			Red	APC*	Alexa Fluor® 647			
			Infra Red	APC-Cy7				

\* APC and PE-Cy5 may be used together on instruments with cross-beam compensation.

## Fluorochrome Specifications

Fluorochrome	Fluorescence Emission Color	Ex-Max (nm)	Excitation Laser Line (nm)	Em-Max (nm)	BD FACScan™	BD FACSCalibur™	BD FACStar Plus™	BD FACSVantage™ SE	BD™ LSR	BD™ LSR II	BD FACSAria™	BD FACSTArray™
Alexa Fluor® 405	Blue	401	360, 405, 407	421				✓		✓	✓	
Pacific Blue®	Blue	410	360, 405, 407	455				✓		✓	✓	
Alexa Fluor® 488	Green	495	488	519	✓	✓	✓	✓	✓	✓	✓	
FITC	Green	494	488	519	✓	✓	✓	✓	✓	✓	✓	
PE	Yellow	496, 546	488, 532	578	✓	✓	✓	✓	✓	✓	✓	✓
PE-Texas Red®	Orange	496, 546	488, 532	615	✓	✓	✓	✓	✓	✓	✓	
Texas Red®**	Orange	595	595	615			✓	✓				
APC*	Red	650	595, 633, 635, 647	660		✓	✓	✓	✓	✓	✓	✓
Alexa Fluor® 647	Red	650	595, 633, 635, 647	668		✓	✓	✓	✓	✓	✓	✓
PE-Cy5*	Red	496, 546	488, 532	667	✓	✓	✓		✓	✓	✓	
PerCP	Red	482	488, 532	678	✓	✓			✓	✓	✓	
PerCP-Cy5.5	Far Red	482	488, 532	695	✓	✓	✓	✓	✓	✓	✓	✓
PE-Cy7	InfraRed†	496, 546	488, 532	785	✓	✓	✓	✓	✓	✓	✓	✓
APC-Cy7	InfraRed†	650	595, 633, 635, 647	785			✓	✓	✓	✓	✓	✓

† InfraRed detection requires a Hamamatsu R3896 Photomultiplier Tube (comes with detector option).

\* APC and PE-Cy5 may be used together on instruments with cross-beam compensation.

\*\* Texas Red® detection requires a dye laser.

## Fluorochrome Notes

Visit [www.bdbiosciences.com/spectra/](http://www.bdbiosciences.com/spectra/) to access an interactive fluorescence spectrum viewer.

Alexa Fluor® 405 dye, with visible-wavelength excitation, has minimal spectral overlap with green fluorophores.

Alexa Fluor® 488 and 647 conjugates are highly photostable and remain fluorescent over a broad pH range.

Allophycocyanin (APC) is an accessory photosynthetic pigment found in bluegreen algae. Its molecular weight is approximately 105 kDa. APC has 6 phycocyanobilin chromophores per molecule, which are similar in structure to phycoerythrobilin, the chromophore in R-PE.

APC-Cy7 is a tandem conjugate system that combines APC and a cyanine dye (Cy7). It is recommended that special precautions be taken with APC-Cy7 conjugates, and cells stained with them, to protect the fluorochrome from long-term exposure to visible light. It is recommended that a 750-nm longpass filter be used along with a red-sensitive detector such as the Hamamatsu R3896 PMT for this fluorochrome.

Fluorescein isothiocyanate (FITC) is a fluorochrome with a molecular weight of 389 daltons. The isothiocyanate derivative (FITC) is the most widely used form for conjugation to antibodies and proteins, but other derivatives are available. FITC has a high quantum yield (efficiency of energy transfer from absorption to emission fluorescence) and approximately half of the absorbed photons are emitted as fluorescent light. The number of FITC molecules per conjugate partner (antibody, avidin, streptavidin, etc.) is usually in the range of three to five molecules.

Pacific Blue® a UV-light excitable dye, is based on the 6,8-difluoro-7-hydroxycoumarin fluorophore and is strongly fluorescent, even at neutral pH.

R-phycoerythrin (R-PE) is an accessory photosynthetic pigment found in red algae. *In vivo*, it functions to transfer light energy to chlorophyll during photosynthesis. *In vitro*, it is a 240-kDa protein with 34 phycoerythrobilin fluorochromes per molecule. The large number of fluorochromes per PE molecule make R-phycoerythrin an ideal pigment for flow cytometry applications.

PE-Cy5 (formerly BD Cy-Chrome™) is a tandem conjugate system which combines R-phycoerythrin (a 240-kDa protein) and a cyanine dye (MW 1.5 kDa). The efficiency of the light energy transfer between the two fluorochromes allows less than 5% of the absorbed light to be lost as fluorescence at 575 nm by R-PE. As with our R-PE conjugates, an average of one PE-Cy5 molecule is coupled per antibody or protein. Because of its broad absorption range, PE-Cy5 is not recommended for use with dual-laser flow cytometers where excitation by both lasers is possible.

PE-Cy7 is a tandem conjugate system that combines PE and a cyanine dye (Cy7). PE-Cy7 can be used simultaneously with APC with minimal crossbeam compensation.

PE-Texas Red® is a tandem conjugate system which combines R-PE and Texas Red. Special care must be taken when using PE-Texas Red conjugates in conjunction with R-PE as there is considerable spectral overlap in the emission profiles of both fluorochromes.

Peridinin chlorophyll protein (PerCP) is a component of the photosynthetic apparatus found in the dinoflagellate, *glenodinium*. PerCP is a protein complex with a molecular weight of approximately 35 kDa. Due to its photobleaching characteristics, PerCP conjugates are not recommended for use on stream-in-air flow cytometers.

PerCP-Cy5.5 is a tandem conjugate system that combines PerCP with a cyanine dye (Cy5.5) . PerCP-Cy5.5 is recommended for use with stream-in-air flow cytometers.

Texas Red® is a sulfonyl chloride derivative of sulforhodamine 101 with a molecular weight of 625 daltons. Texas Red conjugated to avidin, is a useful second step for multi-color analysis. When performing multi-color analysis involving both Texas Red and R-PE, a dual-laser flow cytometer equipped with a tunable dye laser to avoid “leaking” into the PE detector is recommended. If a krypton laser, emitting light at 568 nm, is used, the laser light will “leak” into the R-PE channel. Texas Red can be used in conjunction with APC for multi-color analysis when both dyes are excited in the 595-605 nm range with a dye laser.

### Absorption and emission spectra for BD Biosciences fluorochromes

