

BD Horizon RealBlue™ 780 Reagents

Spectrally optimized laser-specific fluorochrome

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BD Horizon RealBlue™ 780 Reagents

Innovative, laser-specific BD Horizon RealBlue™ 780 (RB780) Reagents offer a unique spectral profile that enables high-resolution data and flexible panel design.

- Primarily excited by the 488-nm blue laser
- Minimal cross-laser excitation off the 561-nm yellow-green laser
- Bright fluorescence to detect low-expression markers
- Lower monocyte background than PE-Cy7
- Can be used in place of or with PE-Cy7 on appropriately configured conventional and spectral flow cytometers

RB780 Specifications		
Ex_{max}	Em_{max}	Relative Brightness
488 nm	780 nm	

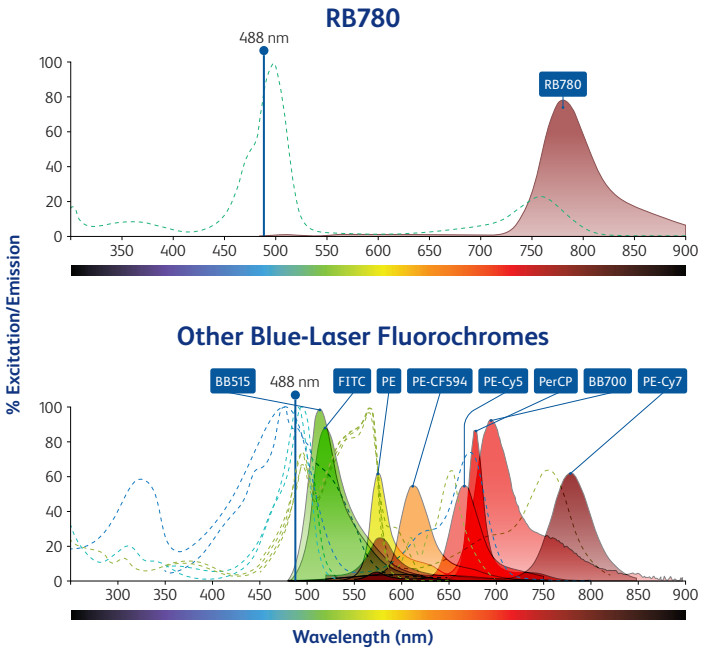


Figure 1. Excitation and emission spectrum for RB780 reagents.

Excitation (dashed lines) and emission (area lines) profiles of RB780 (top) vs. other blue-laser fluorochromes, many of which are cross-excited by the yellow-green laser (bottom). Emission is normalized to laser excitation.

Engineered to help you spend less time optimizing panels and more time discovering

Bright and Clean

Brighter than PE-Cy7 on average.

Minimal nonspecific binding to monocytes.

Low spillover due to minimal cross-laser excitation off the yellow-green laser.

Enables simultaneous use of detectors on the blue and yellow-green laser lines.

Versatile Applications

Supports detection of varying levels of marker expression.

Can be used instead of PE-Cy7 or with PE-Cy7.

Available in a wide range of specificities.

Stable Performance

Photostable reagents with lot-to-lot consistency.

Optimized for Spectral

Facilitates high-parameter experiments with cutting-edge technologies, from spectral flow cytometry to image-based cell sorting.

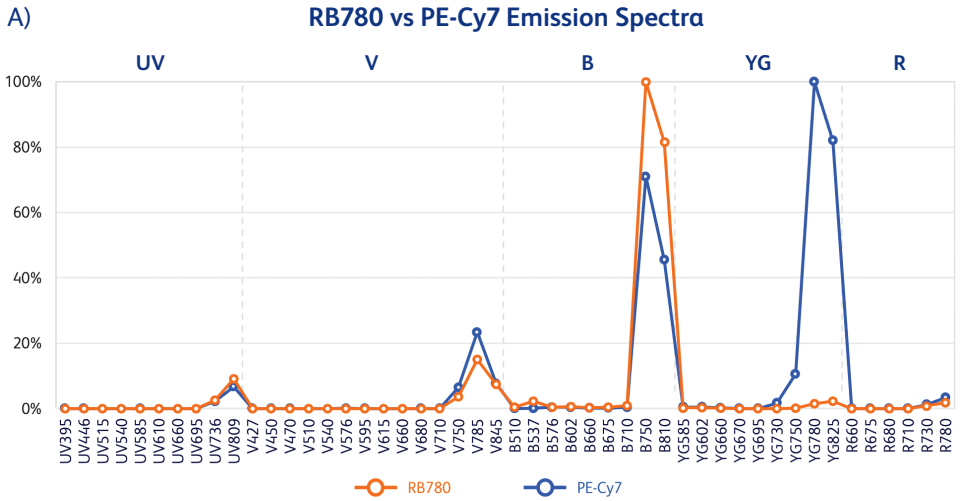
Broadly Compatible

Works well with a variety of common fixation and permeabilization systems.

Bright and Clean

RB780 reagents offer minimal cross-laser excitation and reduced spillover compared to PE-Cy7

A)



B) **RB780 vs PE-Cy7 Spillover into Yellow-Green (YG) Detector**

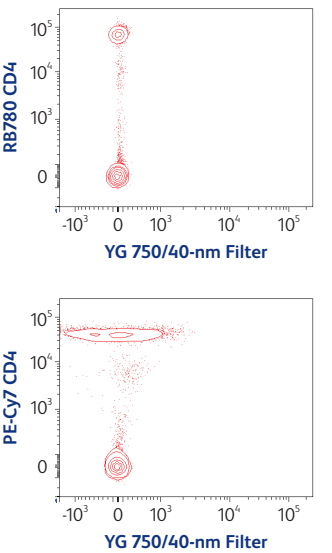


Figure 2. RB780 is primarily excited by the 488-nm laser and shows significantly lower spillover spread into detectors on the yellow-green laser.

A) This chart compares the normalized emission spectra of BD Horizon™ RB780 and PE-Cy7 Reagents. In contrast to PE-Cy7, RB780 reagents display minimal cross-laser excitation off all non-blue lasers, including the yellow-green laser. B) Human whole blood was stained with BD Horizon™ RB780 Human CD4 (SK3) Reagent, acquired on a BD FACSymphony™ A5 SE Cell Analyzer. The biexponential scaling was set for PE-Cy7 and applied to RB780 to highlight changes in spread into selected channels.

The RB780 fluorochrome is very bright and supports the detection of varying levels of marker expression

Brightness of RB780 and Other Reagents

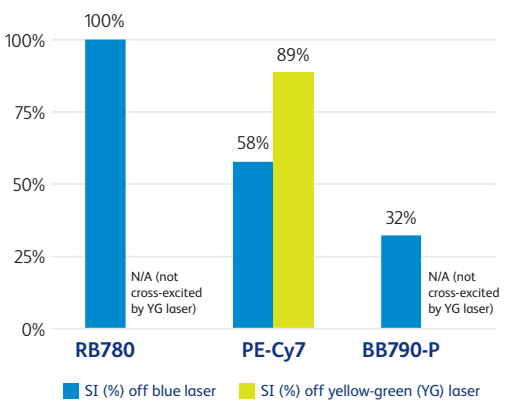


Figure 3. On average, RB780 is brighter than PE-Cy7 and BB790-P.

Stain index (SI) data showing a comparison of BD Horizon™ RB780, PE-Cy7, and BD Horizon™ BB790-P CD4 (SK3) Reagent off the 488-nm blue laser (B810) and PE-Cy7 off the 561-nm yellow-green laser (YG780) in healthy PBMC samples. SI data displayed as a percentage of RB780 SI.

Stain Indices of RB780 and PE-Cy7 Reagents

	RB780 SI	PE-Cy7 SI		RB780 SI (% PE-Cy7 SI)	
	Blue laser	Blue laser	YG laser	Blue laser	YG laser
Human CD3	840	402	507	209%	166%
Human CD4	1,335	790	1,183	173%	113%
Human CD19	430	264	377	163%	114%
Human CD123	269	85	149	318%	181%
Human CD279	11	3	2	319%	550%
Human IFN-g	192	55	56	352%	341%
Mouse CD4	130	121	132	108%	99%
Mouse CD8	114	92	98	124%	117%

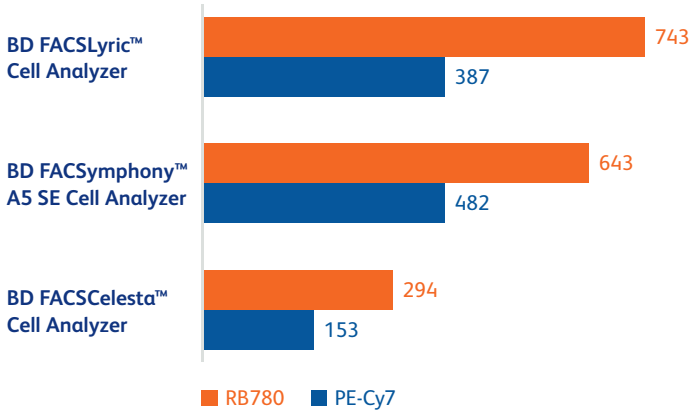
Table 1. RB780 is generally brighter than PE-Cy7 across various clones.

Stain index (SI) data comparing BD Horizon™ RB780 Reagents and PE-Cy7 off the 488-nm blue laser (B810) or 561-nm yellow-green laser (YG780). Table displays RB780 and PE-Cy7 SI values and RB780 SI as a percentage of PE-Cy7 SI. RB780 and PE-Cy7 were used to label multiple markers at optimal concentrations with no unmixing or compensation applied. Data acquired on BD FACSymphony™ A5 SE Cell Analyzer.

Bright and Clean

Outperforms PE-Cy7 in brightness across multiple instruments

A) **RB780 vs PE-Cy7 CD4 Stain Index**



B) **Instrument Configurations**

	Excitation laser (nm)	Laser power (mW)	Filter (nm)
BD FACSLyric™ Cell Analyzer	Blue 488	20	783/56 BP 750 LP
BD FACSymphony™ A5 SE Cell Analyzer	Blue 488	200	810/79 BP 770 LP
	Yellow-green 561	150	780/60 BP 760 LP
BD FACSCelesta™ Cell Analyzer	Blue 488	20	780/60 BP 750 LP

Figure 4. RB780 reagent is shown to be consistently brighter than PE-Cy7.

A) Graph shows stain indices (SI) of PE-Cy7 and BD Horizon™ RB780 CD4 Reagent obtained from PBMC-stained samples, acquired on multiple instruments with different configurations. For PE-Cy7 SI on the BD FACSymphony™ A5 SE Cell Analyzer, data were acquired off the yellow-green laser. For all other SI values, data were acquired off the blue laser. B) Instrument configurations used, including excitation laser wavelength, laser power and bandpass (BP) and longpass (LP) filters.

Significantly less background from nonspecific monocyte binding than PE-Cy7

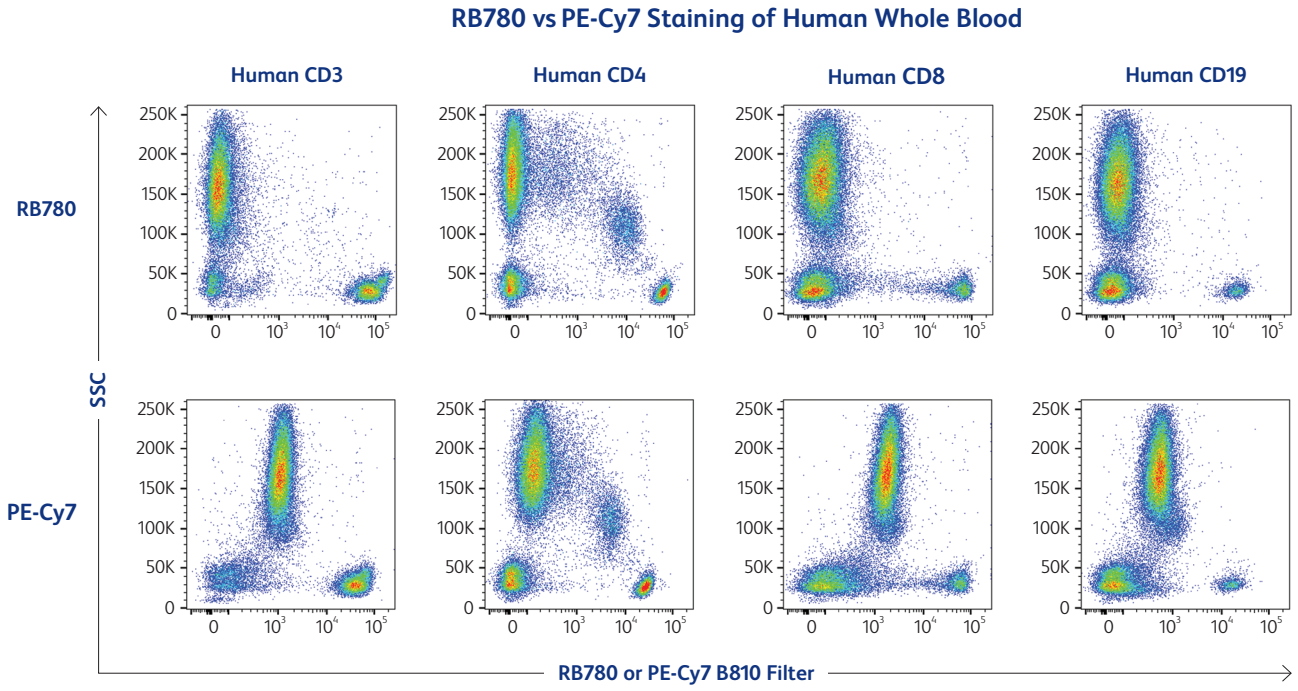


Figure 5. RB780 reagents produce lower background than PE-Cy7, enabling clear resolution of subpopulations.

Human whole blood was stained with PE-Cy7 or BD Horizon™ RB780 Human CD3 (UCHT1), CD4 (SK3), CD8 (RPA-T8), or CD19 (SJ25C1) Reagents, followed by lysis with BD FACS™ Lysing Solution. All specificities were acquired off the blue laser on a BD FACSymphony™ A5 SE Cell Analyzer.

Versatile Applications

RB780 reagents support the detection of a wide variety of specificities, including low-expression surface and intracellular markers

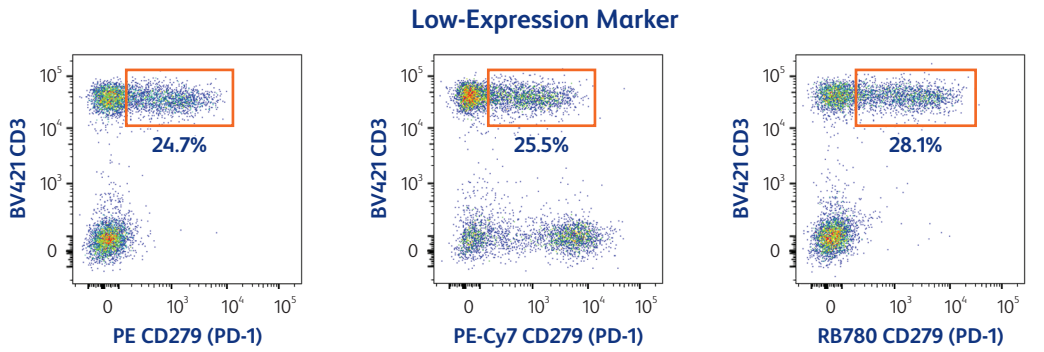


Figure 6. RB780 reagents can be used to detect low-expression surface markers.

Staining of PE, PE-Cy7, and BD Horizon™ RB780 Mouse Anti-Human CD279 (PD-1) Reagent. PE is detected off the yellow-green laser, and PE-Cy7 and RB780 reagents are both detected off the blue laser. Data were acquired and compensated on a BD FACSymphony™ A5 SE Cell Analyzer.

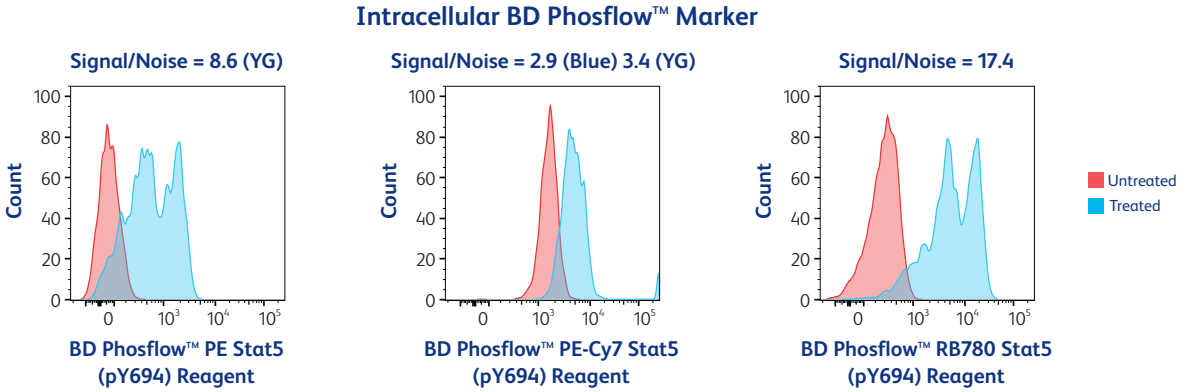


Figure 7. With significantly brighter fluorescence and less background, RB780 reagents enable intracellular markers to be clearly resolved.

Untreated (red) or treated (blue) PBMCs from the BD Phosflow™ T Cell Kit Lyophilized Cells were reconstituted in neutral buffer and then stained with PE (acquired off the yellow-green laser), PE-Cy7 (acquired off the blue laser) or BD Phosflow™ RB780 Stat5 (pY694) (47/Stat5) Reagent. Data were acquired on a BD FACSymphony™ A5 Cell Analyzer.

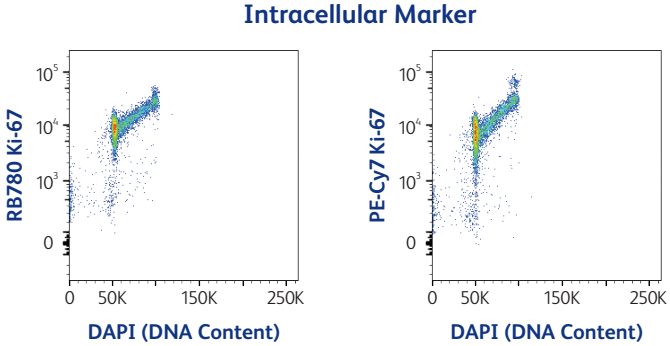


Figure 8. RB780 reagents can resolve challenging intracellular markers.

Molt-4 cells were permeabilized with ice-cold 70% ethanol, stained with BD Horizon™ RB780 Reagent (left) or PE-Cy7 (right, acquired off the blue laser) Ki-67 (B56) and DAPI for DNA content. Data were acquired on a BD FACSymphony™ A5 SE Cell Analyzer with compensation.

Optimized for Spectral

Can be used with PE-Cy7 for multicolor panels and spectral flow cytometry

Spectral Unmixing of RB780 and PE-Cy7 CD4

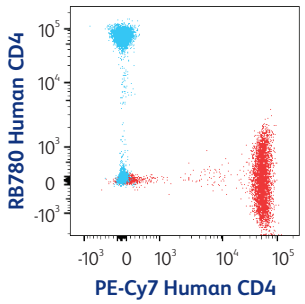


Figure 9. RB780 reagents and PE-Cy7 can be used together in spectral flow cytometry.

Bivariate overlay plots of spectrally unmixed single-color CD4 (with either RB780 or PE-Cy7) stained PBMCs obtained from healthy donor. Samples were acquired on a BD FACSymphony™ A5 SE Cell Analyzer and spectrally unmixed using FlowJo™ v10.8 Software.

RB780 and PE-Cy7 Reagents Used in a Multicolor Panel to Identify Rare Cell Populations

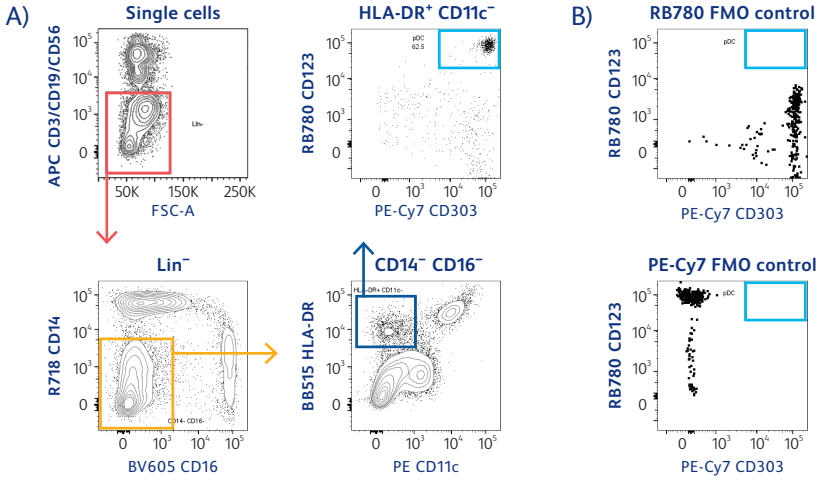


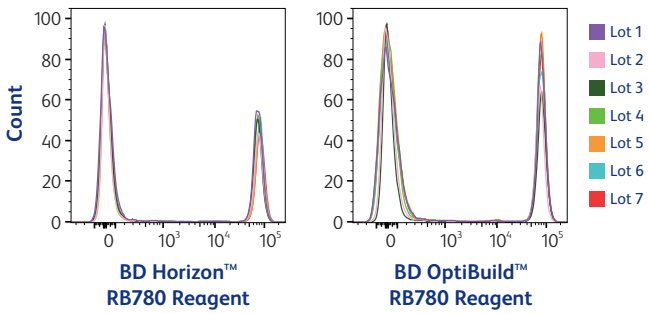
Figure 10. RB780 and PE-Cy7 reagents were used to detect a pDC subpopulation.

A) Flow plots show clear distinction of the pDC population marked as CD123$^+$ CD303$^+$ cells derived from lineage-negative (Lin$^-$) CD14$^-$ CD16$^-$, HLA-DR$^+$ CD11c$^-$ cell populations in healthy PBMC samples. Samples were stained with the panel reagents along with Human BD Fc Block™ Reagent, analyzed on a BD FACSymphony™ A5 SE Cell Analyzer and spectrally unmixed using FlowJo™ v10.8 Software. B) FMO Controls used for gating show the minimal spread of RB780 into PE-Cy7.

Stable Performance

Proven lot-to-lot consistency across multiple specificities

Human CD4



Mouse CD8a

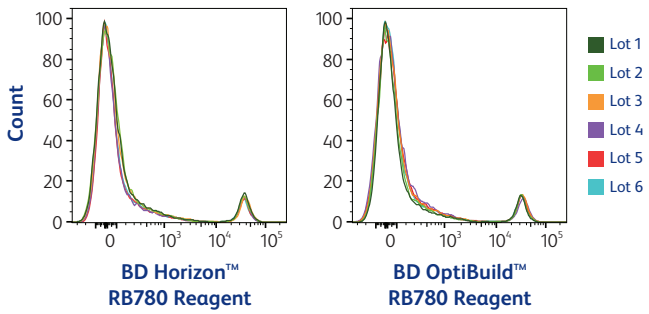


Figure 12. RB780 reagents demonstrate lot-to-lot consistency across made-to-stock and BD OptiBuild™ On-Demand Reagents.

Human whole blood was stained with BD Horizon™ or BD OptiBuild™ RB780 Human CD4 (Clone SK3) Reagent using seven different lots of reagent, followed by lysis with BD FACS™ Lysing Solution. Mouse splenocytes were stained with mouse CD8a (53-6.7) using six different lots of reagent. Data were acquired on a BD FACSymphony™ A5 SE Cell Analyzer. All reagent lots were made within two months of each other.

Broadly Compatible

Compatible with a broad range of fixation and permeabilization systems

Buffers	Results
BD FACS™ Lysing Solution and BD Pharm Lyse™ Lysing Buffer	Compatible
CellBlox™ Blocking Buffer	Compatible
BD Cytofix™ Fixation Buffer	Stable at least 24 hours
1% PFA	Stable at least 24 hours
BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution	Compatible with antibody staining before and after fixation
BD FACS™ Permeabilizing Solution II	Compatible with antibody staining before and after fixation
BD Phosflow™ Perm III	Compatible with antibody staining before and after fixation
EDTA and heparin	Compatible
BD Horizon™ Brilliant Stain Buffer (BSB)	Compatible

FAQs

Can RB780 reagents be used with PE-Cy7 in a panel on a conventional flow cytometry instrument?

Yes, if the instrument has appropriate filters for PE-Cy7 on both the blue and yellow-green lasers.

Can RB780 reagents be used together with PE-Cy7 on a BD LSRFortessa™ Cell Analyzer?

No, not in the standard configuration.

What is the size of the RB780 fluorochrome?

Less than 30 kDa.

Are RB780 reagents based on polymer technology?

RB780 reagents use a proprietary next-generation tandem dye technology that is different from Sirigen polymer technology.

Do RB780 reagents need special buffers or handling to prevent dye-to-dye interactions?

No. However, for human whole blood specimens we recommend using BD Horizon™ Brilliant Stain Buffer (BSB) to minimize possible background that may be caused by anti-PEG antibodies.

Are RB780 reagents compatible with viability dyes?

Yes, RB780 reagents are compatible with all viability dyes.

BD Horizon RealYellow™ and RealBlue™ Reagents

A family of bright, laser-specific fluorochromes that simplify panel design and improve data resolution even for the most complex analysis.

Cross-laser excitation can complicate panel design and data analysis, slowing down your research. Spend less time optimizing panels and more time discovering with the family of BD Horizon RealBlue™ and RealYellow™ Reagents.

This bright, clean laser-specific family of fluorochromes offers minimal cross-laser excitation and less spillover to help maximize panel flexibility for both conventional and spectral flow cytometry.

Streamline your path to scientific breakthrough with BD Horizon RealYellow™ and RealBlue™ Reagents.

BD flow cytometers are Class 1 Laser Products.

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