

BD Horizon RealYellow™ 586 Reagents

Spectrally optimized laser-specific fluorochrome

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BD Horizon RealYellow™ 586 Reagents

BD Horizon RealYellow™ 586 (RY586) Reagents use an innovative, laser-specific fluorochrome. They are excited primarily by the 561-nm yellow-green laser and offer:

- Minimal cross-laser excitation off the 488-nm blue laser
- Bright fluorescence to detect low-expression markers

With a unique spectral profile, RY586 reagents can be used with PE on spectral flow cytometers to increase parameters for deep scientific insights or used instead of PE on conventional flow cytometers for flexible panel design.

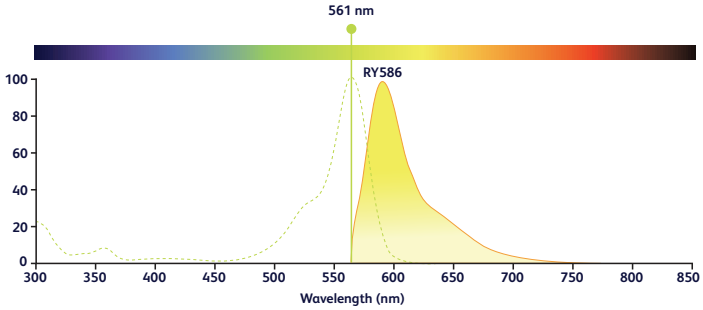

Figure 1. Excitation and emission spectrum of RY586.

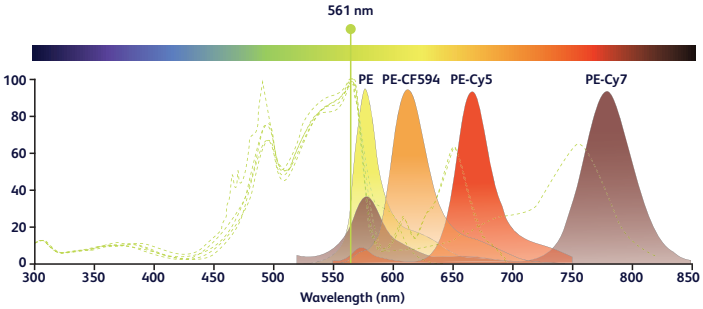

Figure 2. Excitation and emission spectrum of PE and PE tandem dyes.

RY586 Specifications

Ex_{max}	Em_{max}	Relative Brightness
565 nm	586 nm	

BD Horizon RealYellow™ 586 Reagents are engineered to help you spend less time optimizing panels and more time discovering.

Bright and Clean

Bright fluorochrome to support the detection of markers with varying levels of expression.

Minimal background.

Minimal cross-laser excitation off the blue, violet and UV lasers.

Less spillover spread into multiple blue channels and select violet and UV channels compared to PE.

Versatile

Supports detection of surface and intracellular markers.

Can be used instead of PE on conventional flow cytometers or with PE on spectral flow cytometers.

Available in a wide range of specificities to support a variety of applications.

Spectral

Specially designed to enable high-parameter panel design for spectral flow cytometry.

Stable

Lot-to-lot consistency.

Photostable.

Compatible

Compatible with a variety of common fixation and permeabilization systems.

Bright & Clean

BD Horizon RealYellow™ 586 Reagents offer minimal cross-laser excitation off the blue, violet and UV lasers.

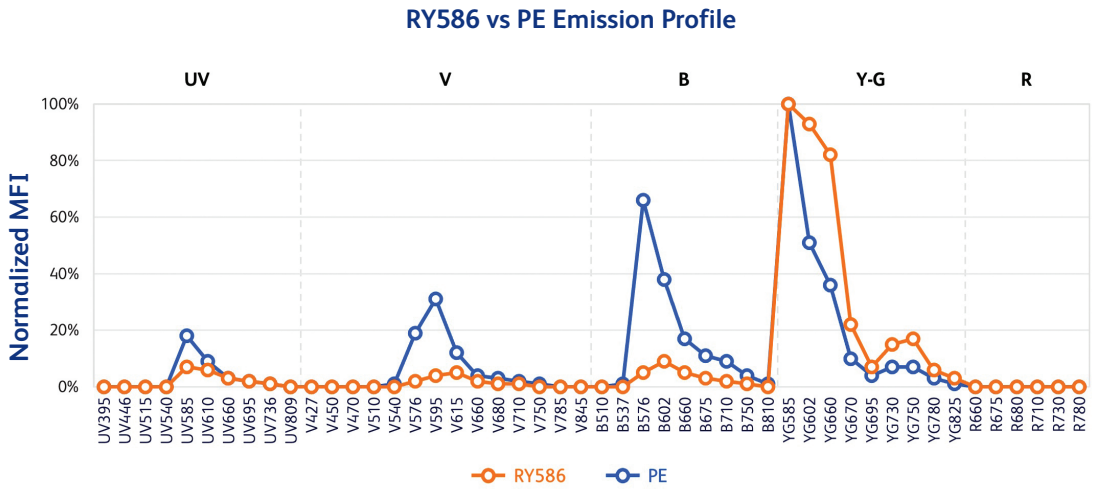

Figure 3. RY586 exhibits significantly less cross-laser excitation by the blue, violet and UV lasers, resulting in less spillover.

This chart compares the normalized emission profile of PE and RY586, showing lower emission of RY586 into blue, violet and UV detectors.

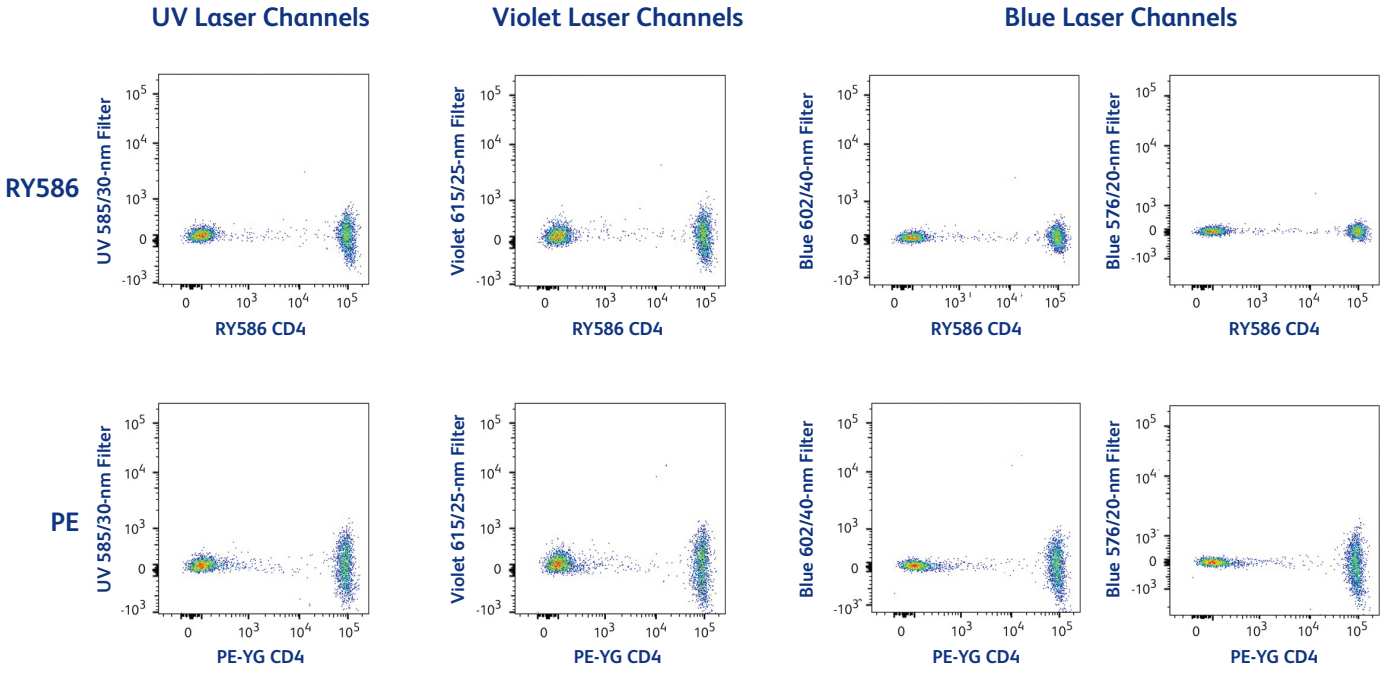

Figure 4. RY586 has less spillover spread into blue, violet and UV laser channels than PE.

Human whole blood was stained with BD Horizon™ RY586 Human CD4 (SK3) Reagent, acquired and compensated on a BD FACSymphony™ A5 SE Cell Analyzer.

Bright & Clean

RY586 is a bright fluorchrome designed to enhance the resolution of varying levels of marker expression.

RY586 Brightness vs Other Reagents

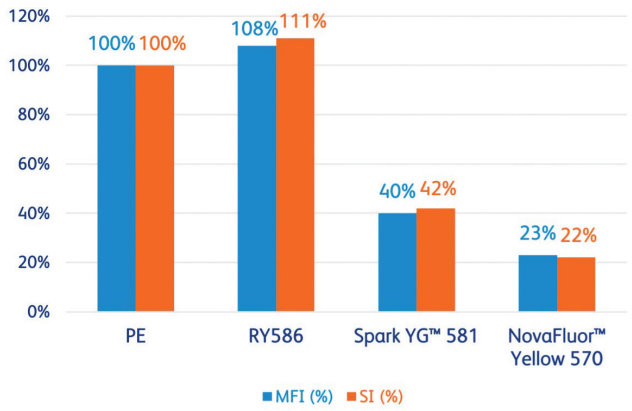

Figure 5. RY586 is brighter than PE and other fluorochromes.

Mean fluorescence intensity and stain index data show that RY586 CD4 is brighter than PE, Spark YG™ 581 and NovaFluor™ Yellow 570 CD4 reagents. Similar trends were seen for other specificities.

RY586 vs PE Stain Index for Various Markers

	RY586 Stain Index	PE Stain Index	%PE
Human CD3	676	486	139%
Human CD19	391	476	82%
Mouse CD4	114	90	127%
Mouse CD8	67	49	137%

Table 1. RY586 is generally comparable to or brighter than PE across multiple clones.

Comparison of RY586 and PE stain index data based on excitation by the 561-nm yellow-green laser.

RY586 offers minimal background and high resolution comparable to PE.

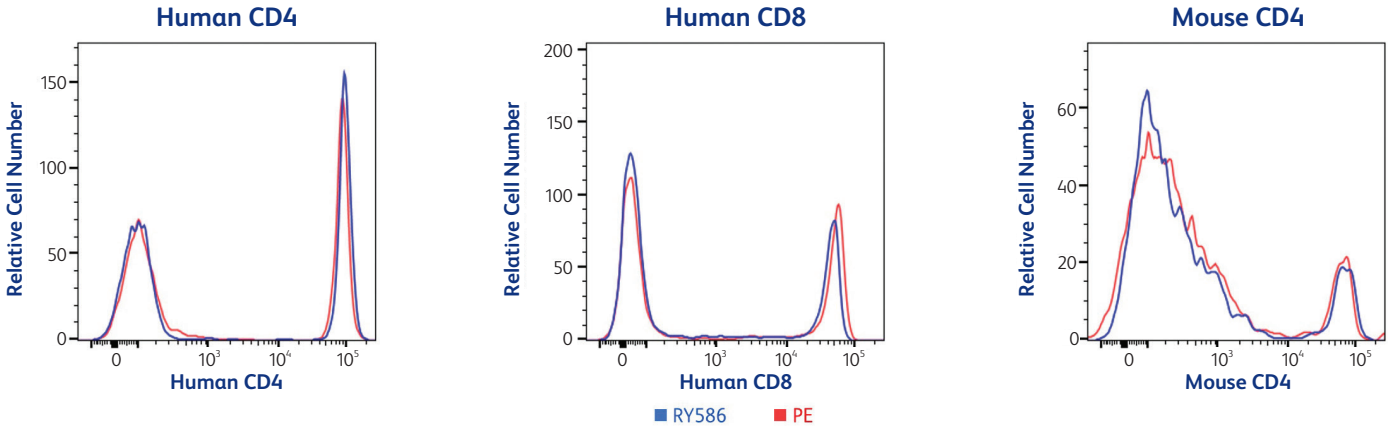

Figure 6. RY586 has comparable background to PE based on spread and MFI of the negative population.

Human whole blood was stained with human CD4 (SK3), human CD8 (RPA-T8) PE or RY586, followed by lysis with BD FACS™ Lysing Solution. Mouse splenocytes were stained with mouse CD4 (GK1.5) PE (acquired off the yellow-green laser) or RY586. All specificities were performed on a BD FACSymphony™ A5 SE Cell Analyzer.

Versatile Applications

RY586 reagents support the detection of various antigens, including low-expression surface and intracellular markers.

Low-Expression Marker

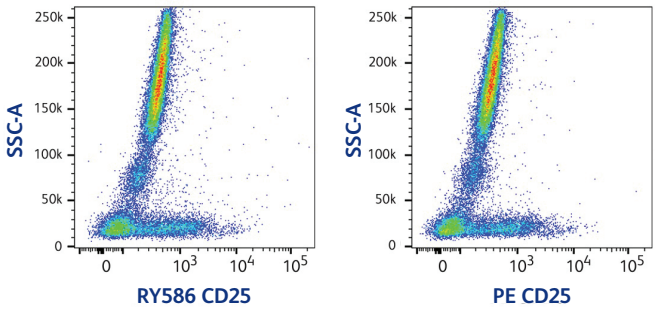

Figure 7. Low-expression surface markers can be resolved comparably with PE and RY586.

Human whole blood was stained with RY586 (left) or PE (right, acquired off the yellow-green laser) CD25 (2A3) and acquired on a BD FACSymphony™ A5 SE Cell Analyzer.

Intracellular Cytokine Marker

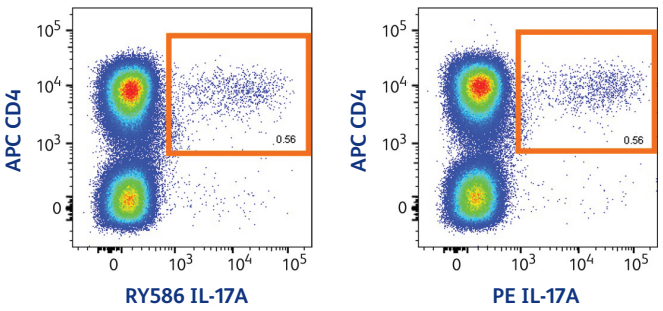

Figure 8. Like PE, RY586 delivers clear resolution of intracellular markers.

Human PBMCs were stimulated with PMA and ionomycin in the presence of BD GolgiStop™ Protein Transport Inhibitor for 5 hours and then stained with APC CD4 and either RY586 (left, 0.125 µg) or PE (right, 0.25 µg, acquired off the yellow-green laser) IL-17a (N49-653) and acquired on a BD LSRFortessa™ Cell Analyzer.

Use RY586 instead of PE on conventional flow cytometers for more flexible panel design or with PE on spectral flow cytometers for high-dimensional analysis.

RY586 vs PE on Conventional Flow Cytometer

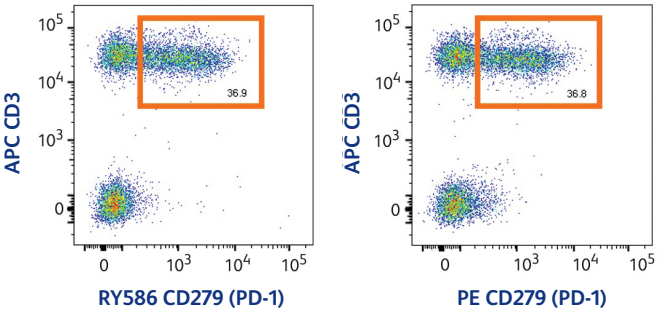

Figure 9. RY586 delivers comparable resolution to PE and minimal cross-laser excitation on a conventional flow cytometer.

Staining of PE and BD Horizon™ RY586 Mouse Anti-Human CD279 (PD-1) Reagent, both detected off the yellow-green laser. Data acquired and compensated on a BD FACSymphony™ A5 SE Cell Analyzer and gated by FMO.

RY586 and PE on a Spectral Flow Cytometer

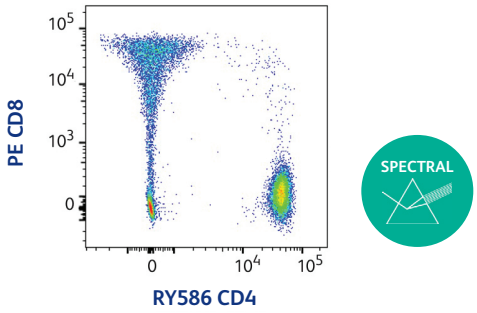

Figure 10. RY586 and PE can be clearly differentiated from each other on a spectral flow cytometer.

Human PBMCs co-stained with PE and BD Horizon RY586 reagents. Data acquired and spectrally unmixed on a BD FACSymphony™ A5 SE Cell Analyzer.

RY586 is available in a wide range of antibody specificities and clones.

Multicolor Panel for Spectral

RY586 and PE in the same multicolor spectral flow cytometry panel enable the resolution of distinct populations.

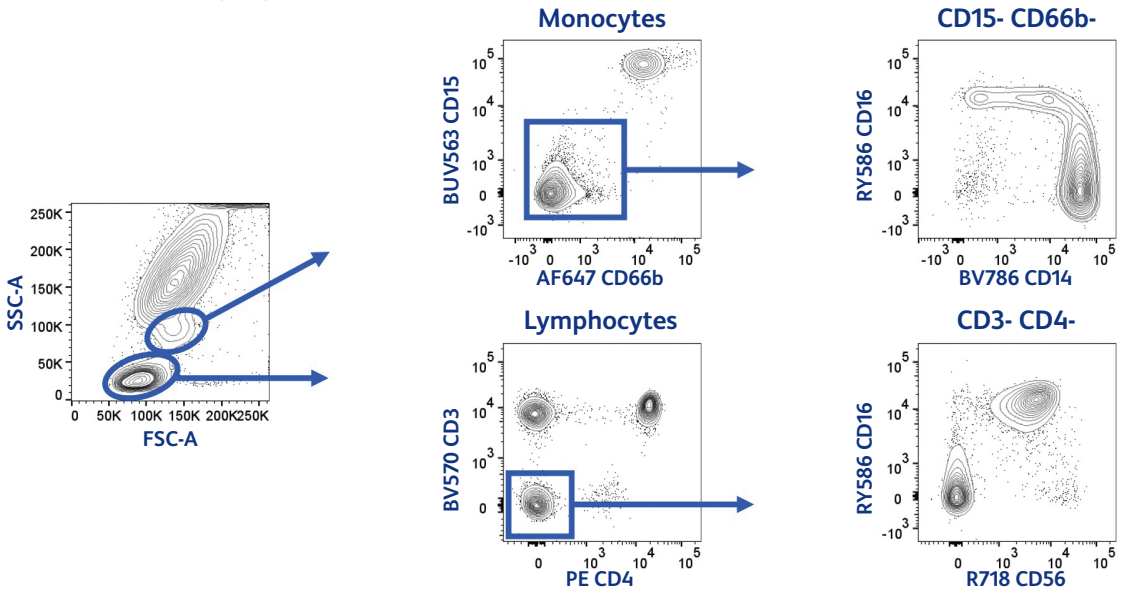

Figure 11. Using both RY586 and PE enables this multicolor panel to measure CD16 expression in specific human immune cell populations.

A multicolor panel using both RY586 and PE fluorochromes to characterize human immune cells expressing different levels of CD16. Cells were acquired on a BD FACSymphony™ A5 SE Cell Analyzer and analyzed with FlowJo™ Software. Spectral unmixing matrix contained 14 fluorochromes.

Consistent and Stable

RY586 offers stable reagent performance with lot-to-lot consistency.

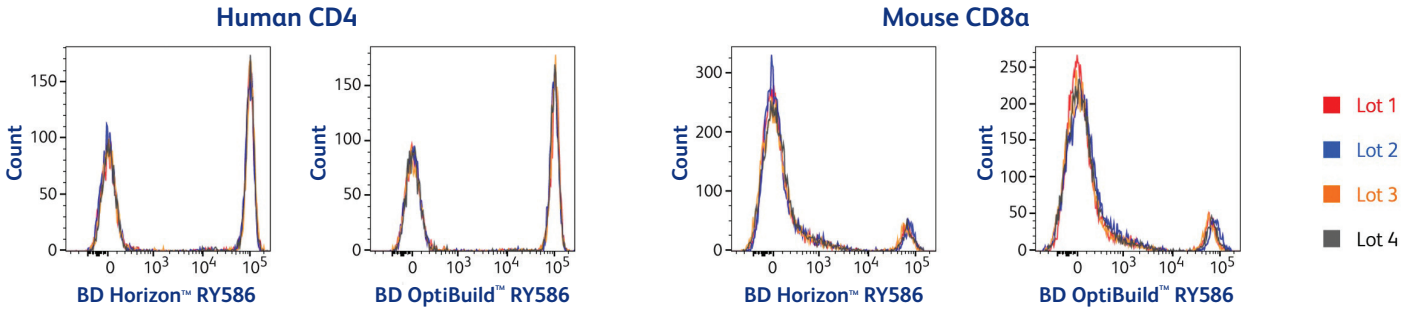

Figure 12. RY586 lot-to-lot consistency is demonstrated across made-to-stock and BD OptiBuild™ On-Demand Reagents.

Human whole blood was stained with human CD4 (SK3) RY586, followed by lysis with BD FACS™ Lysing Solution. Mouse splenocytes were stained with mouse CD8a (53-6.7). All specificities were run on a BD FACSymphony™ A5 SE Cell Analyzer.

RY586 is photostable when exposed to typical lab lighting or dimmed core lab lighting.

Wide Compatibility

RY586 reagents are compatible with a broad range of common 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 RY586 be used with the 532-nm green laser instrument configuration?

No. For optimal results, please use a yellow laser (561 nm) configuration. For the 532-nm green laser configuration, we recommend using PE.

Can RY586 be used with PE in a panel on a conventional flow cytometry instrument?

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

Is RY586 based on polymer technology?

No, RY586 is a specially engineered small molecule organic fluorochrome.

What is the size of RY586?

It is a small organic molecule (<10 kDa).

Does RY586 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.

Is RY586 compatible with viability dyes?

Yes, RY586 is compatible with viability dyes. However, not recommended for use with Fixable Viability Stain 570 due to spectral overlap.

BD Horizon RealYellow™ and RealBlue™ Reagents

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

Ambiguous data can slow down your path to discovery. BD Horizon RealYellow™ and RealBlue™ Reagents use next-generation fluorochrome technology driven by BD innovation, including AI-guided fluorochrome selection for optimal spectral positioning. These new reagents are designed to have laser-specific excitation profiles and narrow emission profiles that maximize resolution and minimize spillover when used with other fluorochromes so you can expand your research with ease on both conventional and spectral flow cytometers.

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

BD flow cytometers are Class 1 Laser Products.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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