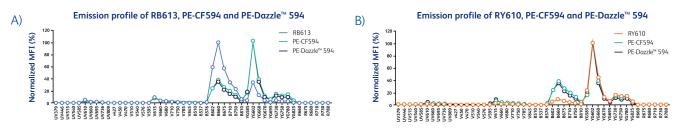
BD Horizon RealBlue<sup>™</sup> 613 (RB613) and BD Horizon RealYellow<sup>™</sup> 610 (RY610) Reagents are members of a family of reagents specially engineered to deliver reduced spillover and optimize resolution when used with other fluorochromes—helping to enable flexible panel design and high-parameter research on both conventional and spectral flow cytometers. Compared to PE-tandem fluorochromes such as PE-CF594 and PE-Dazzle<sup>™</sup> 594, these bright fluorochromes are designed to offer reduced cross-laser excitation, provide stable reagent performance for reproducible results and be compatible with a variety of common fixation and permeabilization systems.

Format	Laser Line	Instrument	Cross-laser excitation	 Alternative to
RB613	488-nm blue	spectral + conventional	reduced off the 561-nm yellow-green	PE-CF594 or PE-Dazzle™ 594 when used with a blue laser*
RY610	561-nm yellow-green	spectral + conventional	minimal off the 488-nm blue	PE-CF594 or PE-Dazzle™ 594 when used with a yellow-green laser**

<sup>\*</sup> On three-laser configuration (B, V, R)

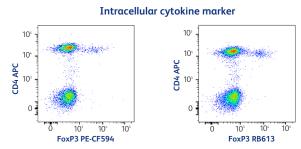
#### RB613 and RY610 have reduced cross-laser excitation



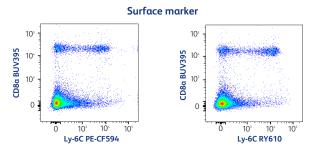
#### Normalized emission profiles of RB613, RY610, PE CF594 and PE-Dazzle™ 594 Fluorochromes

RY610 and RB613 have distinct emission profiles and reduced cross-laser excitation. A and B) Samples run on a BD FACSymphony™ A5 SE Cell Analyzer.

### A variety of expression levels can be resolved with RB613 and RY610



Human PBMCs were fixed and permeabilized using the BD Pharmingen™ Transcription Factor Buffer Set. Cells were then stained with PE-CF594 (left) or RB613 (right) FoxP3 (259D/C7) and costained with CD4 (SK3) APC followed by acquisition on a BD FACSymphony™ A5 SE Cell Analyzer with compensation in FlowJo™ Software. Data shown using the B602 filter for both PE-CF594 and RB613.



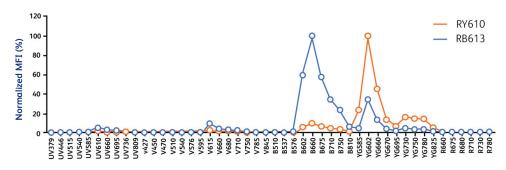
Mouse splenocytes were stained with PE-CF594 (left) or BD Horizon™ RY610 Reagent (right) Ly-6C (AL-21), co-stained with BUV395 CD8a (53-6.7) and acquired on a BD FACSymphony™ A5 SE Cell Analyzer with compensation.



<sup>\*\*</sup> On four- or five-laser configuration (B, V, R, YG or UV, V, B, YG, R)

# RB613 and RY610 can be used together in flow cytometry panels

RB613 and RY610 have distinct spectral profiles so that they can be used together on instruments with both blue and yellow-green lasers and the appropriate filters, such as the BD FACSymphony<sup> $\mathrm{IM}$ </sup> A3, A5, and A5 SE Analyzers as well as the BD FACSDiscover<sup> $\mathrm{IM}$ </sup> S8 Cell Sorter.



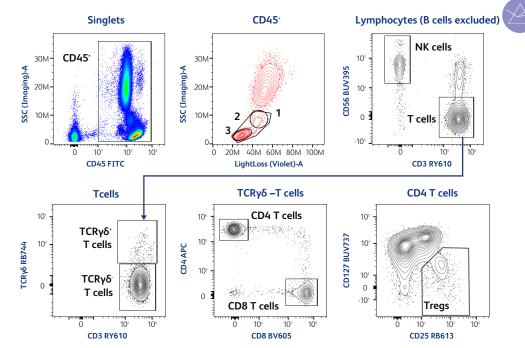
## Normalized emission profiles for RB613 and RY610

RY610 and RB613 have distinct emission profiles and minimal cross-laser excitation. Samples run on a BD FACSymphony™ A5 SE Cell Analyzer.

Here we've demonstrated the use of RB613 and RY610 together in a 22-color human lineage spectral panel run on the BD FACSDiscover $^{\text{\tiny{M}}}$  S8 Cell Sorter.

# RB613 and RY610 reagents work well together in a 22-color human lineage spectral panel

Human peripheral fresh whole blood samples were stained with antibodies against cell surface markers prior to bulk red blood cell lysis with BD Pharm Lyse™ Lysing Buffer. Cells were then analyzed on a BD FACSDiscover™ S8 Cell Sorter and analyzed with FlowJo™Software v10.9. A gating strategy for detection of NK and T cell subsets, after exclusion of doublets and B cells, is shown. In the CD45<sup>+</sup> population, population 1 is referring to monocytes only, 2 is referring to monocytes and lymphocytes and 3 is referring to lymphocytes only.





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