BD




# BD Horizon RealYellow™ and RealBlue™ Reagents

## Designed to minimize spillover spread and improve panel resolution

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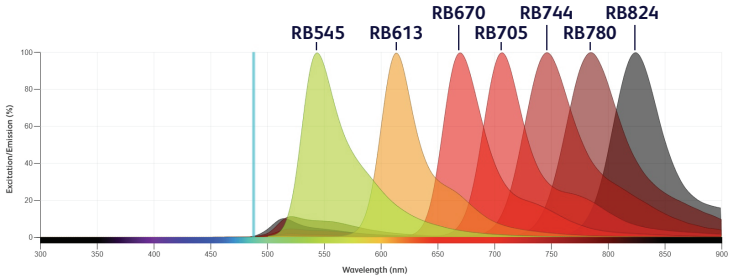
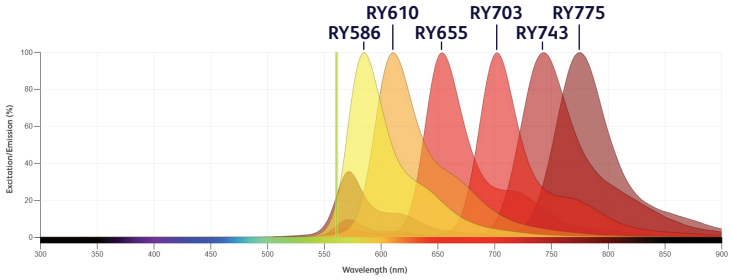
# BD Horizon RealYellow™ and RealBlue™ Reagents

Modern flow cytometers are equipped with multiple lasers and advanced detectors, enabling the use of a broad spectrum of fluorescent dyes. However, this has increased the issue of spillover spread where fluorescent emission signals "spill" into the primary detectors of other fluorochromes. This is especially apparent with PE and PE tandem fluorochromes that are excited by both the blue and yellow-green lasers.

As instruments have become more sophisticated, the need for fluorescent dyes that can more easily be used together in complex flow cytometry panels has grown. BD Horizon RealYellow™ and RealBlue™ Reagents were designed to meet this need. These fluorochromes are laser-specific to help simplify panel design, improve data resolution and increase the number of detectable parameters in a single sample.




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

### Bright and Clean

Bright fluorochromes to support the detection of markers with varying levels of expression

Minimal background

Reduced spillover compared to legacy fluorochromes

### Versatile

Supports detection of surface and intracellular markers

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

### Spectral

Designed to enable higher parameter panel design for spectral flow cytometry

### Stable

Lot-to-lot consistency for reproducibility

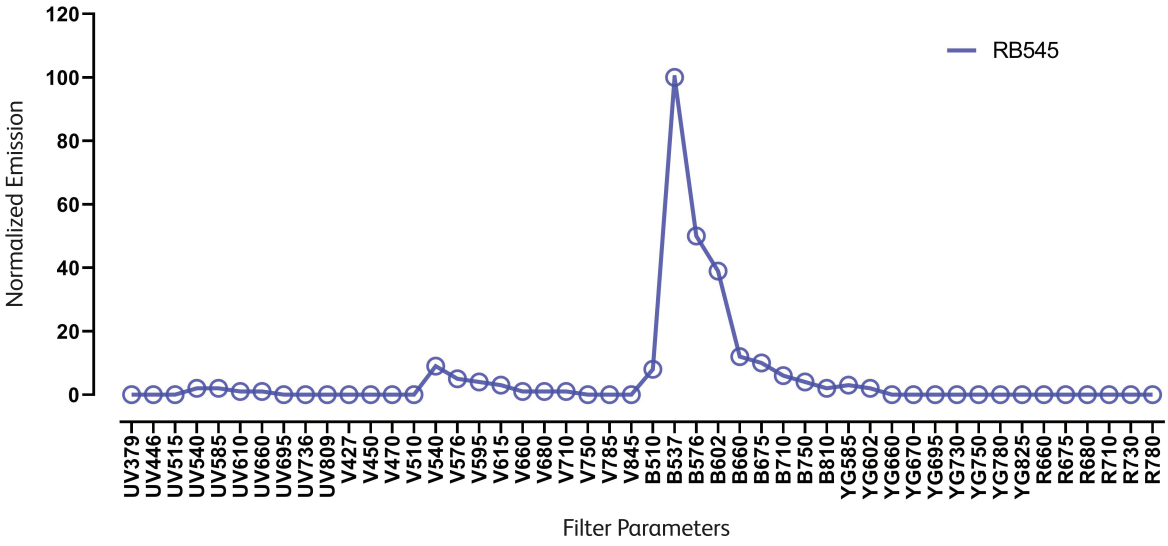
Photostable

### Compatible

Compatibile with a variety of common fixation and permeabilization systems

# Bright and Clean

## RealYellow™ and RealBlue™ Reagents offer reduced spillover from cross-laser excitation

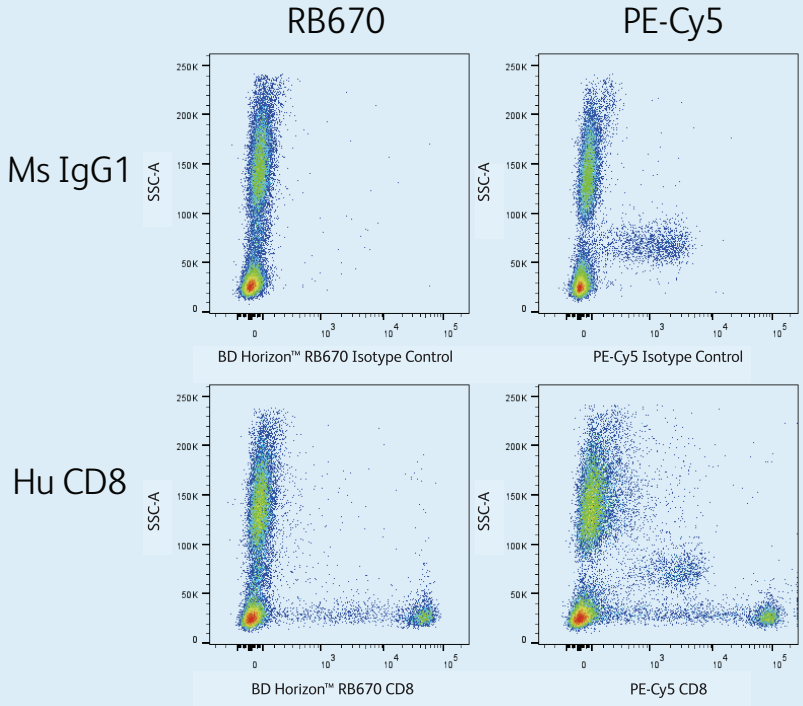



### BD Horizon RealBlue™ 545 (RB545) Reagents offer minimal cross-laser excitation

Normalized emission profile of the RB545 reagent with minimal excitation. Acquired on a BD FACSymphony™ A5 SE Cell Analyzer.

### BD Horizon RealBlue™ 670 (RB670) Reagents have reduced monocyte background compared to PE-Cy5

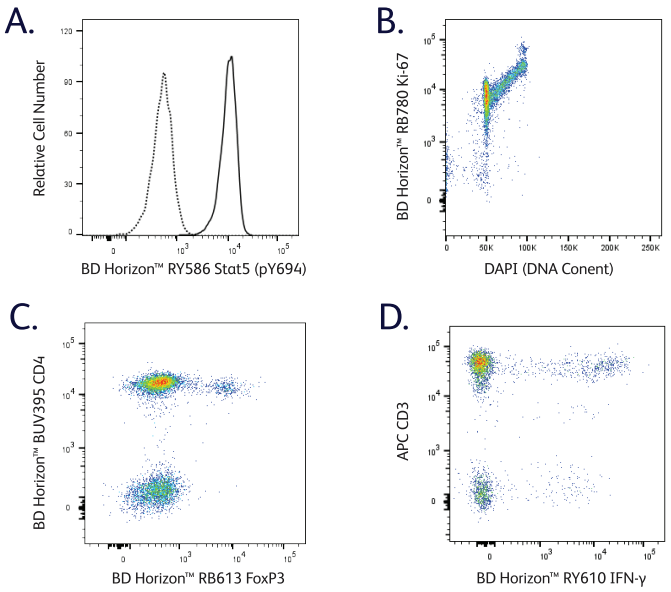
Human PBMCs were stained with RB670 or PE-Cy5 Human CD8 (RPA-T8) and mouse IgG1 isotype control (X40), followed by erythrocyte lysis using BD Pharm Lyse™ Lysing Buffer, and acquired on a BD FACSymphony™ A5 SE Cell Analyzer and analyzed with FlowJo™ Software.



# Versatile Applications

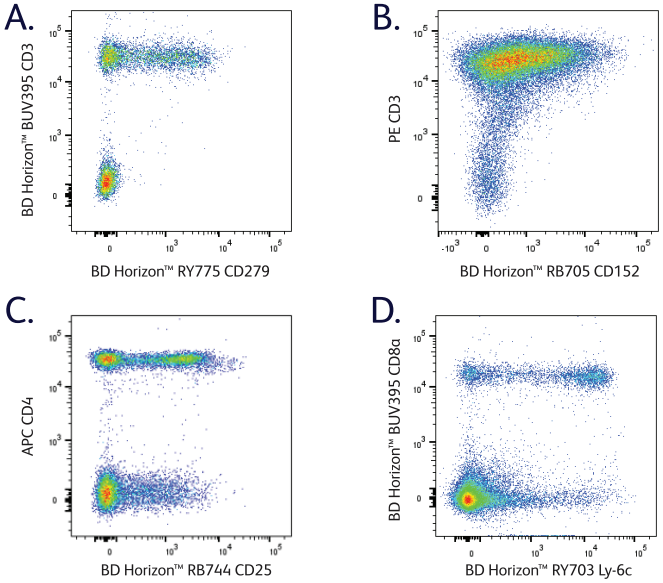
## RealYellow™ and RealBlue™ Reagents support the detection of varying levels of antigen expression, including low-expression surface and intracellular markers

### RealYellow™ and RealBlue™ Reagents work well for intracellular markers




A) Untreated (dotted line) or treated (solid line) PBMCs from the BD Phosflow™ T Cell Kit. Lyophilized Cells were reconstituted in neutral buffer and then stained with BD Horizon™ RY586 Stat5 (pY694) (47/Stat5). B) Molt-4 cells were permeabilized with ice-cold 70% ethanol, stained with BD Horizon™ RB780 Reagent Ki-67 (B56) and DAPI for DNA content. C) Human PBMCs were fixed and permeabilized using the BD Pharmingen™ Transcription Factor Buffer Set. Cells were then stained with BD Horizon™ RB613 FoxP3 (259D/C7) and costained with APC CD4 (SK3). D) PBMCs were stimulated for 5 hours with PMA and Ionomycin in the presence of BD GolgiStop™ Protein Transport Inhibitor, and fixed with BD Cytofix™ Fixation Buffer. Cells were surface stained with CD3 APC, followed by permibolization and intracellular staining with BD Horizon™ RY610 IFN-γ (B27). A-D) All samples were acquired on a BD FACSymphony™ A5 SE Cell Analyzer and analyzed in FlowJo™ Software.

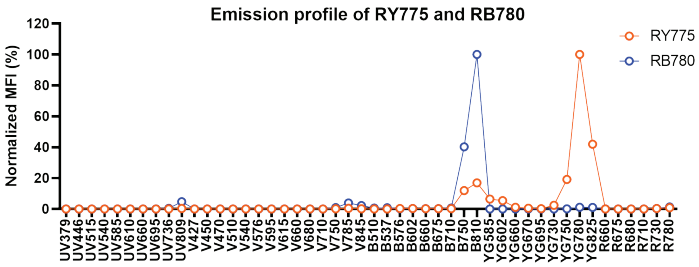
### Low-to-moderate antigen expression markers are well resolved with RealYellow™ and RealBlue™ Reagents




A) Human whole blood was stained with BD Horizon™ RY775 CD279 (EH12.1) and APC CD3 (UCHT1). B) PHA-activated human PBMCs were stained with PE CD3 (UCHT.1) and BD Horizon™ RB705 CD152 (CTLA-4) (BNI3) antibodies. C) Human whole blood was stained with APC CD4 (RPA-T4) and BD Horizon™ RB744 Mouse Anti-Human CD25 ( IL-2 Receptor α) (2A3) antibodies. D) Mouse splenocytes were stained with BD Horizon™ BUV395 CD8 (53-6.7) and BD Horizon™ RY703 Ly-6C (AL-21) antibody. A-D) All samples acquired on a BD FACSymphony™ A5 SE Cell Analyzer with compensation in FlowJo™ Software.

# Optimized for Spectral

## RealYellow™ and RealBlue™ Reagents can be used together in spectral flow cytometry panels



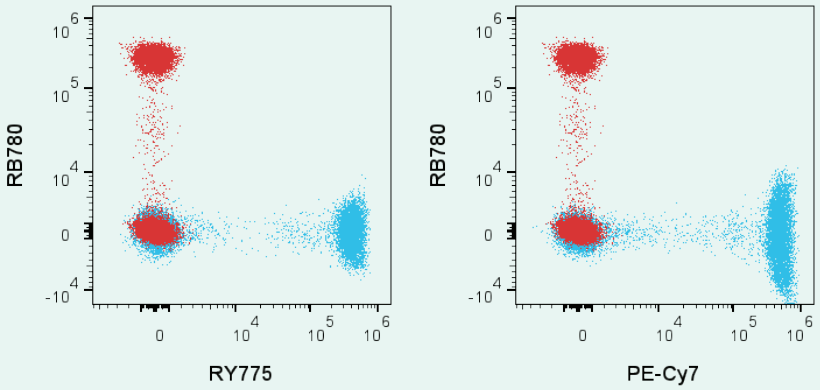

### Normalized emission profiles for BD Horizon RealBlue™ 780 (RB780) and RealYellow™ 775 (RY775)

RB780 and RY775 have distinct emission profiles and minimal cross-laser excitation. Samples were run on a BD FACSymphony™ A5 SE Cell Analyzer.

For improved performance and increased panel design flexibility, RY775 reagents can be paired with RB780 reagents. Both dyes have reduced cross-laser excitation compared to PE-Cy7, which enables their superior performance when used together on instruments with both blue and yellow-green lasers and the appropriate filters, such as five-laser BD FACSymphony™ Analyzers and spectral flow cytometers. The same holds true for the other BD Horizon RealYellow™ and RealBlue™ Reagent family members.

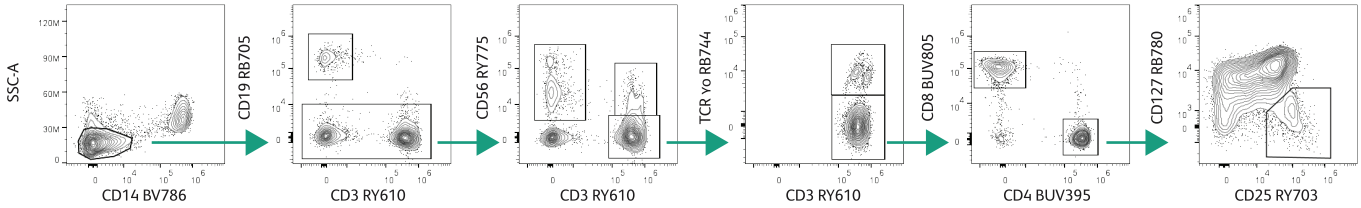
### Pair RB780 with RY775 instead of PE-Cy7 for more flexibility in panel design

PBMCs were stained with either RB780, RY775 or PE-Cy7 CD4, acquired on the BD FACSDiscover™ S8 Cell Sorter and spectrally unmixed using FlowJo™ Software. Resulting data were overlaid to show spread.



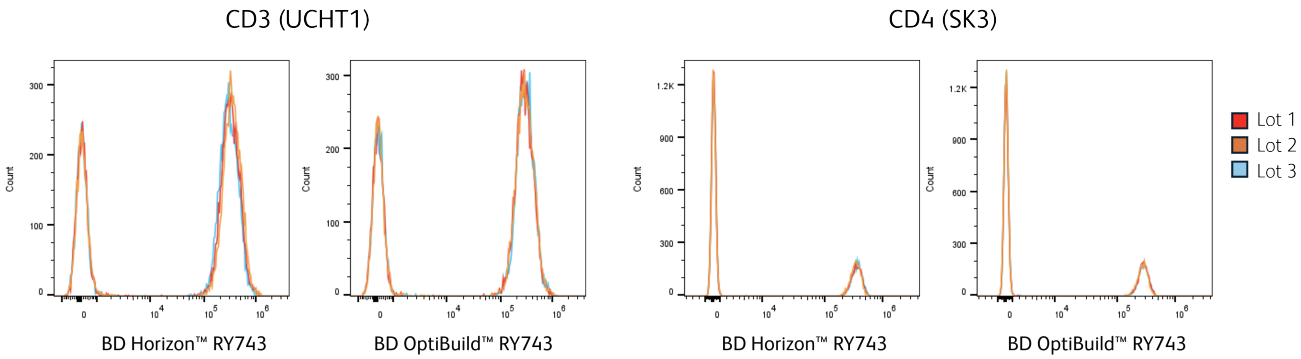

### RY775 and RB780 reagents work well together in an 18-color spectral human immunophenotyping panel

Human PBMCs were stained with a viability dye and antibodies against cell surface markers. Cells were analyzed on a BD FACSDiscover™ S8 Cell Sorter and data were analyzed with FlowJo™ Software v10.10. A gating strategy for the detection of B cells and NK and T-cell subsets, after exclusion of dead cells and doublets, is shown.



# Stable Performance

## Proven lot-to-lot consistency across multiple specificities




### RY743 reagents demonstrate lot-to-lot consistency across made-to-stock and BD OptiBuild™ On-Demand Reagents

Human whole blood was stained with three different lots of either human CD3 (UCHT1, left) or CD4 (SK3, right) RY743, followed by lysis with BD Pharm Lyse™ Lysing Solution. All specificities were run on a Cytek® Aurora Spectral Analyzer. CD3 and CD4 were tested in separate experiments.

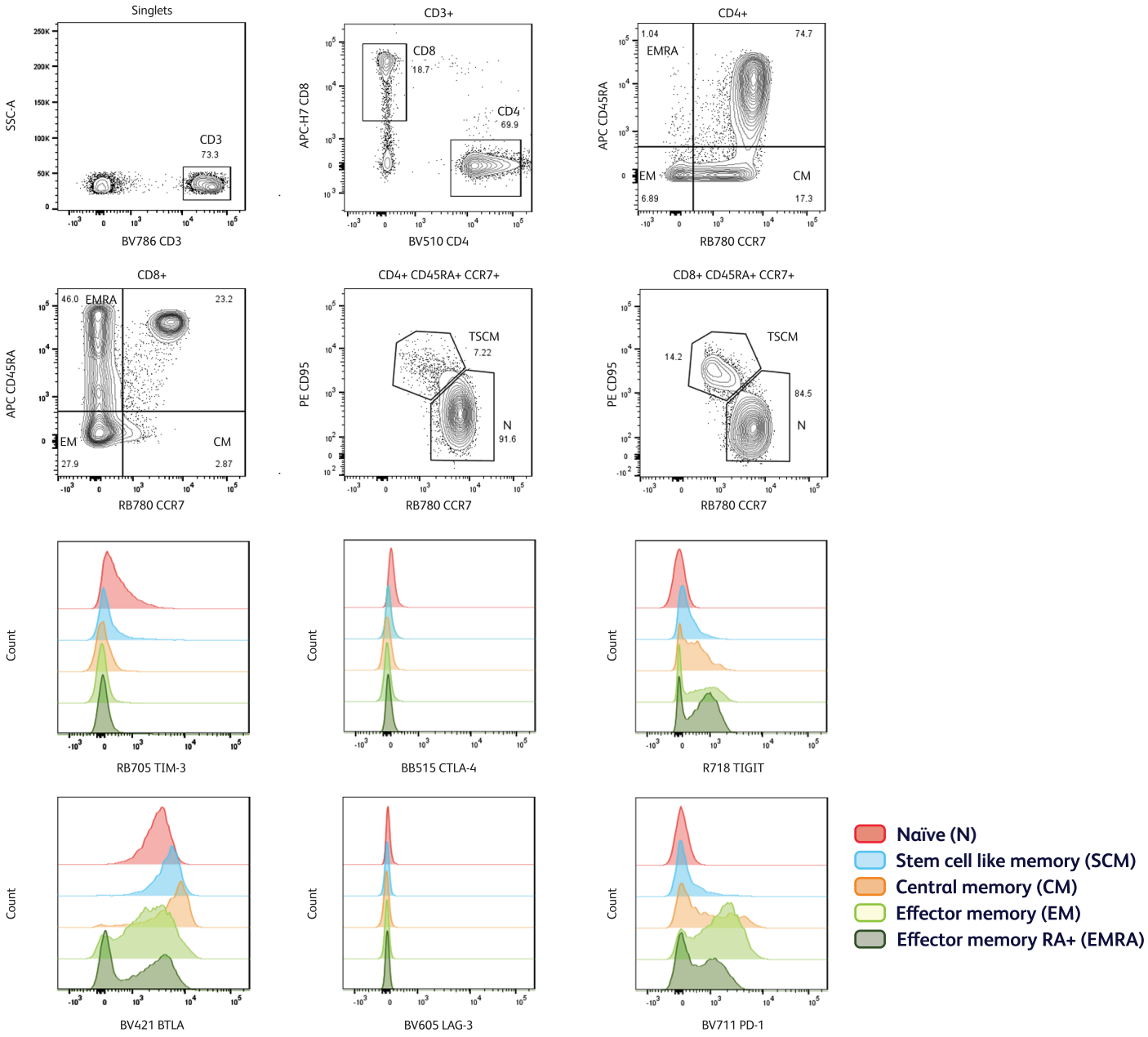
# Broadly Compatible

## Compatible with a broad range of fixation and permeabilization systems

| Buffers | Results |
|---|---|
| **BD FACS™ Lysing Solution and BD Pharm Lyse™ Lysing Buffer** | Compatible |
| **BD Cytofix™ Fixation Buffer** | Stable at least 24 hours |
| **1% PFA** | Stable at least 24 hours |
| **BD Cytofix/Cytoperm™ Fixation and Permabilization Solution** | Compatible with antibody staining before and after fixation |
| **BD FACS™ Permabilizing Solution 2** | Compatible with antibody staining before and after fixation |
| **BD Phosflow™ Perm Buffer III** | Compatible with antibody staining before and after fixation |
| **EDTA and heparin** | Compatible |
| **BD Horizon™ Brilliant Stain Buffer (BSB)** | Compatible |

# Broadly Compatible

## Suitable as alternatives for legacy PE and PerCP tandem dyes run on conventional flow cytometers




Healthy PBMC were stained with a 12-color inhibitory (EMRA) T cell panel and acquired on the BD FACSLyric™ Cell Analyzer. Labels on top of each plot show the parental gate from which plots are derived; numbers in each quadrant represent the frequency of cell population; T cell subsets are identified as N, naïve; SCM, stem cell like memory; CM, central memory; EM, effector memory; and EMRA, effector memory RA+. Histogram overlays depict expression pattern of inhibitory receptors TIM-3, CTLA-4, TIGIT, BTLA, LAG-3 and PD-1 on each of the subsets representative of CD8+ T naïve, stem cell memory, central memory, effector memory and effector memory RA. Data were run on a BD FACSLyric™ Cell Analyzer and analyzed with FlowJo™ Software.

# Cleaner Fluorochromes Are Here

**Swap out your PerCP and PE tandem dyes with RealYellow™ and ReaBlue™ Reagents. These reagents are engineered to deliver reduced spillover and optimize resolution when used with other fluorochromes—helping to enable flexible panel design on both conventional and spectral flow cytometers.**

| Format | Spectral | Conventional | Relative Brightness | Spillover (1 = low, 4 = high) | Alternative to |
|---|---|---|---|---|---|
| **Blue Laser** | | | | | |
| RB545 | ✓ | | ●●○○ | 1 | **(Unique position)** |
| RB613 | ✓ | ✓ | ●●●● | 2 | **PE-CF594, PE-Dazzle™ 594, BB630-P2 or StarBright™ Blue 615** |
| RB670 | ✓ | ✓ | ●●●● | 2 | **PE-Cy5, PerCP, BB660-P2 or StarBright™ Blue 675** |
| RB705 | ✓ | ✓ | ●●●● | 2 | **PerCP-Cy5.5, BB700 or StarBright™ Blue 700** |
| RB744 | ✓ | ✓ | ●●●● | 1 | **BB755-P** |
| RB780 | ✓ | ✓ | ●●●● | 1 | **PE-Cy7 or BB790-P** |
| RB824 | ✓ | ✓ | ●●●○ | 1 | **PE/Fire™ 806 or StarBright™ Blue 810** |
| **Yellow-green Laser** | | | | | |
| RY586 | ✓ | ✓ | ●●●● | 1 | **PE** |
| RY610 | ✓ | ✓ | ●●●○ | 1 | **PE-CF594 or PE-Dazzle™ 594** |
| RY655 | ✓ | ✓ | ●●●● | 3 | **PE-Cy5 or StarBright™ Yellow 665** |
| RY703 | ✓ | ✓ | ●●●● | 2 | **PE-Cy5.5 or StarBright™ Yellow 720** |
| RY743 | ✓ | ✓ | ●●●● | 2 | **PE/Fire™ 744** |
| RY775 | ✓ | ✓ | ●●●● | 2 | **PE-Cy7 or StarBright™ Yellow 800** |

Note: PE and PE tandem dyes are excited by both the blue and yellow-green lasers. Be sure to check your instrument configuration. Spillover column refers to cross-laser spillover.

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