

BD Horizon™ Red 718 Reagents

Enabling greater experimental insights with the power of BD Horizon™ Red 718 Reagents

Features

- Greater resolving power than Alexa Fluor® 700
 - Brighter signal
 - Reduced background
- Excellent resolution of intracellular and surface markers
- Minimal spillover emission into the APC channel
- Lot-to-lot consistency

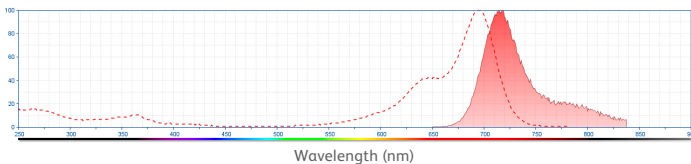



Figure 1. Excitation and emission spectra of R718 Reagents

| R718 Specifications | | |
|---------------------|-------------------|---|
| Ex _{max} | Em _{max} | Relative Brightness |
| 695 nm | 718 nm |  Bright |

When increasing the number of parameters in a multicolor experiment, running more than two colors off the red laser may become necessary. BD Horizon™ Red 718 (R718) Reagents were developed exclusively for BD Biosciences in order to increase the utility of the third red channel. R718 is a small-molecule dye excited by the red (640 or 628 nm) laser with an emission maximum of 718 nm. R718 can be detected in the same filter as Alexa Fluor® 700 and can be used as an alternative to Alexa Fluor® 700 or APC-R700. Due to its improved brightness and low background, R718 offers greater resolving power on surface and intracellular markers compared to Alexa Fluor® 700. Furthermore, this enables the resolution of low expression markers without having to switch to a tandem dye, which can introduce residual emission resulting in spillover/spread into the APC channel.

BD Horizon™ Red 718 Reagents Deliver

Performance: Bright organic dye with low background

Confidence: Clearly resolve antigens with low expression levels

Convenience: Hundreds of reagents available through BD OptiBuild™ On-Demand Reagents

Reliability: Lot-to-lot consistency and reagent stability

| | BD Horizon™ Red 718 Stain Index | Alexa Fluor® 700 Stain Index | X-fold Increase |
|---------|---------------------------------|------------------------------|-----------------|
| Hu CD3 | 373 | 131 | 2.8 |
| Hu CD4 | 297 | 89 | 3.3 |
| Hu CD19 | 59 | 32 | 1.8 |
| Ms CD4 | 89 | 58 | 1.5 |
| Ms CD8α | 106 | 51 | 2.1 |

Table 1. BD Horizon™ Red 718 Reagents have a higher stain index than Alexa Fluor® 700.

Reagents of the same clone were run side by side to compare the stain indices.

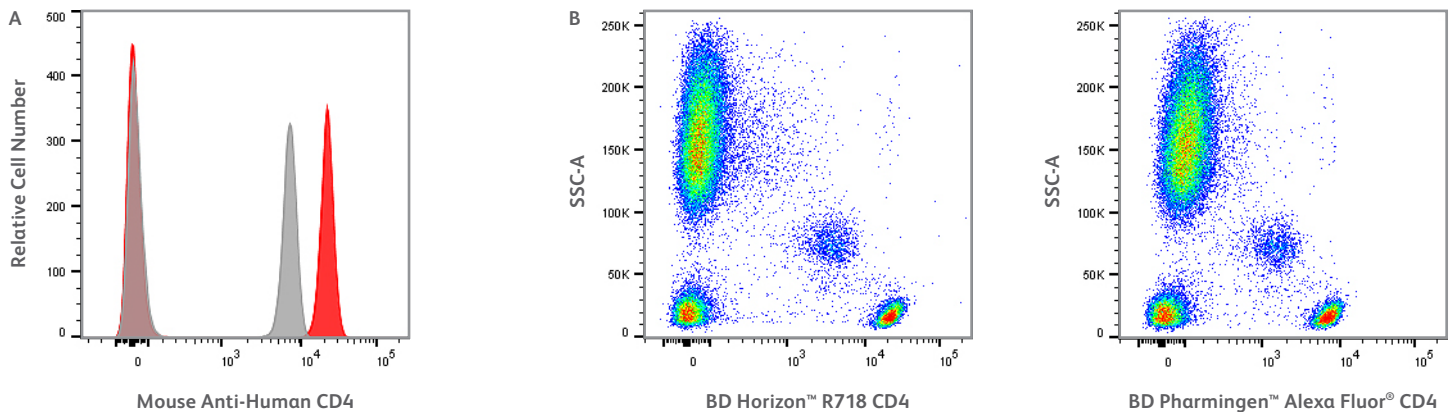


Figure 2. BD Horizon™ R718 Reagent shows superior brightness and resolution as compared to Alexa Fluor® 700.

Comparison staining of CD4 on human lysed whole blood. A. Lysed whole blood stained with BD Horizon™ R718 Mouse Anti-Human CD4 (clone SK3, red) or BD Pharmingen™ Alexa Fluor® 700 Mouse Anti-Human CD4 (clone SK3, grey) shown overlaid as fluorescence histograms derived from a lymphocyte gate. B. SSC vs. fluorescence plots of BD Horizon™ R718 Mouse Anti-Human CD4 (left) or BD Pharmingen™ Alexa Fluor® 700 Mouse Anti-Human CD4 (right). Data were analyzed on a BD LSRFortessa™ X-20 Cell Analyzer with FlowJo™ Software.

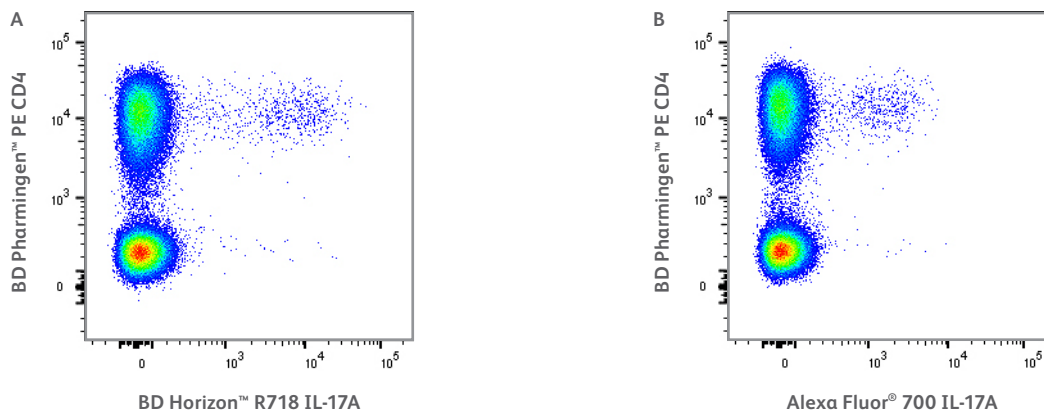


Figure 3. The CD4*IL-17+ cells are better resolved with BD Horizon™ R718 Reagents than Alexa Fluor® 700.

Two-color comparison staining of IL-17A on stimulated human peripheral blood lymphocytes. Human peripheral blood mononuclear cells were stimulated for 5 hours with Phorbol 12-Myristate 13-Acetate and Ionomycin in the presence of BD GolgiStop™ Protein Transport Inhibitor. The cells were permeabilized with BD Perm/Wash™ Buffer then stained with BD Horizon™ R718 Mouse Anti-Human IL-17A (clone N49-653, A), or BD Pharmingen™ Alexa Fluor® 700 Mouse Anti-Human IL-17A (clone N49-653, B). Data shown as two-color flow cytometric pseudocolor density plots derived from gated lymphocytes for BD Pharmingen™ PE Mouse Anti-Human CD4 (clone RPA-T4). Data were analyzed the same day on a BD LSRFortessa™ X-20 Cell Analyzer with FlowJo™ Software.

BD Horizon™ R718 Reagents Resolve Intracellular Markers

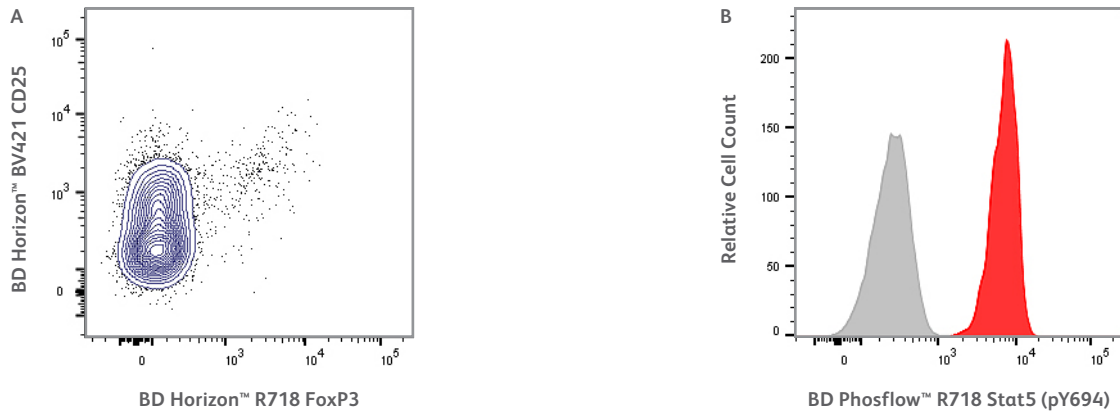


Figure 4. BD Horizon™ R718 Reagent intracellular staining of FoxP3 and Stat5.

BD Horizon R718 resolves difficult intracellular markers, expanding the capabilities of the 700 nm detector. A. intracellular Mouse Anti-Human FoxP3 (clone 259D/C7) staining on human PBMCs with BD Horizon™ R718 and co-stained with BD Horizon™ BV421 Mouse Anti-Human CD25 (clone M-A251). B. BD Phosflow™ Treated Human Control Cells (red) and BD Phosflow™ Untreated Human Control Cells (grey) were stained with BD Phosflow™ R718 Mouse Anti-Stat5 (pY694) (clone 47); treated and untreated control cell overlays shown here are derived from a CD4⁺ gate.

BD Horizon™ R718 Reagents Offer Less Spread into the APC Detector

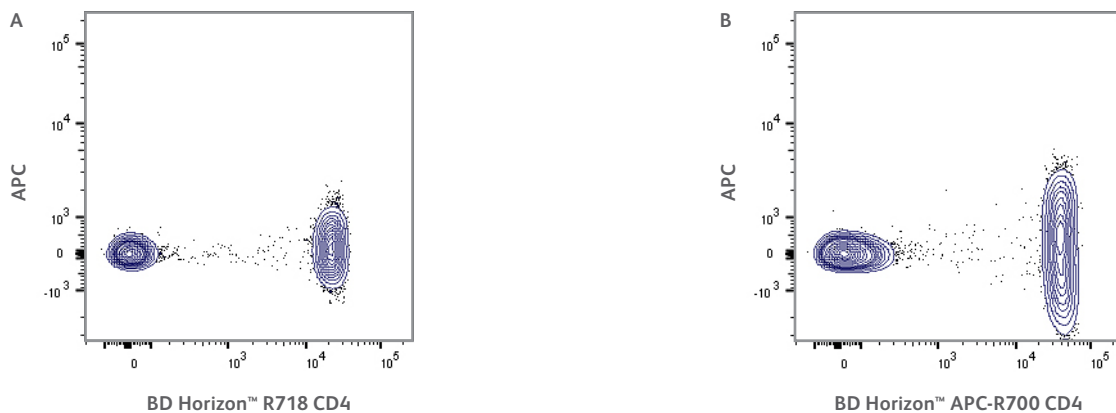


Figure 5. Relatively low spread was observed from BD Horizon™ R718 Reagents when compared to BD Horizon™ APC-R700 in the APC detector.

Lysed whole blood was stained with mouse anti-human CD4 (clone SK3) antibodies conjugated with (A) BD Horizon™ R718 and (B) BD Horizon™ APC-R700. Both reagents were analyzed on the same day on a BD LSRFortessa™ X-20 Cell Analyzer and analyzed with FlowJo™ Software.

8-Color Interleukin Panel with BD Horizon™ R718 Mouse Anti-Human IL-4 (clone MP4-25D2)

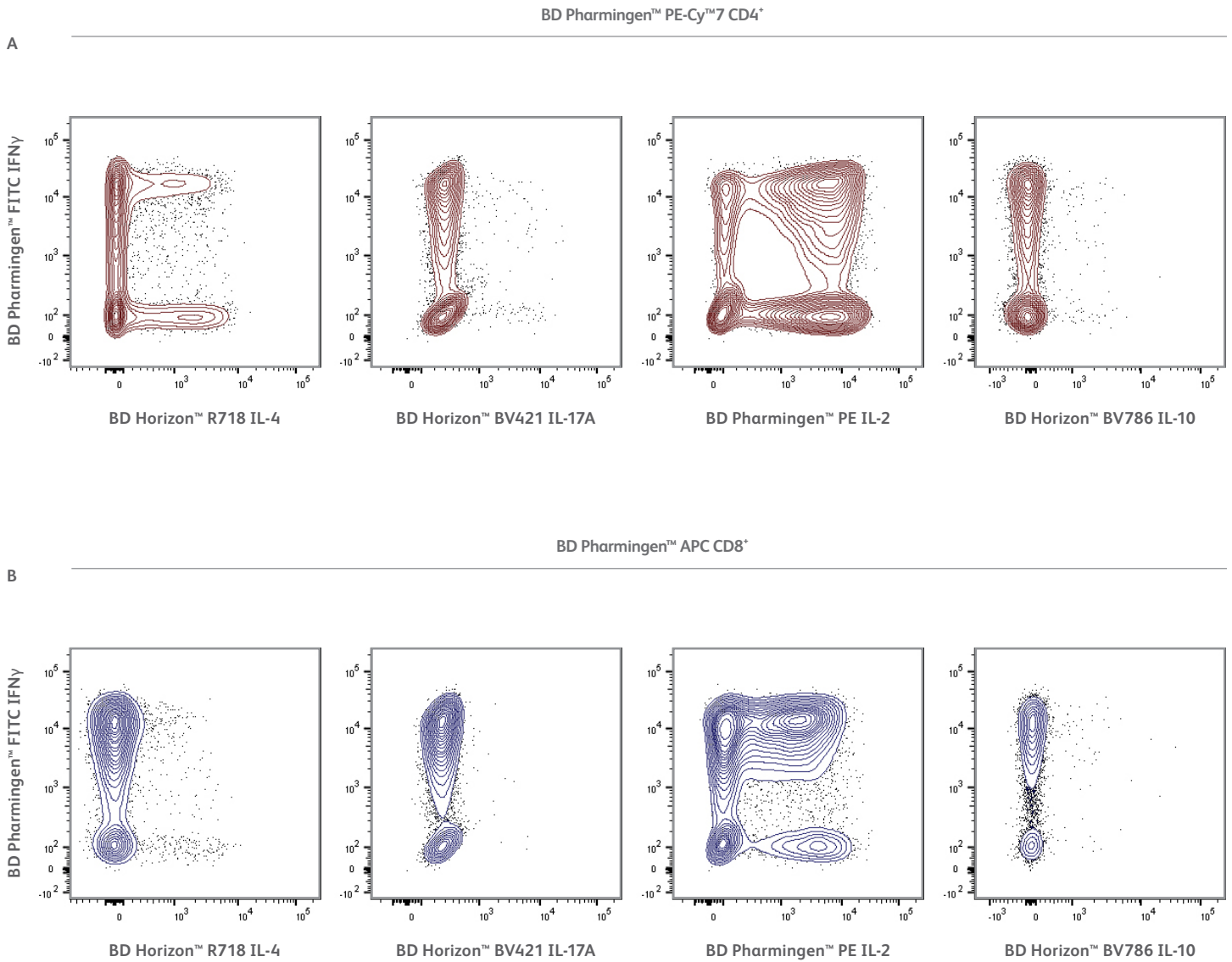


Figure 6. BD Horizon™ R718 Reagents demonstrate excellent resolution of IL-4 as shown in left plots for both CD8⁺ and CD4⁺ T cells in a human T-cell cytokine response panel.

Human PBMCs were stimulated with immobilized purified NA/LE mouse anti-human CD3 (plate bound), soluble purified NA/LE mouse anti-human CD28, recombinant human IL-2 and human IL-4 for 2 days. The cells were subsequently cultured in medium containing recombinant human IL-2 and human IL-4 for 3 days. Finally, the cells were harvested and stimulated for 5 hours with phorbol 12-myristate 13-acetate and ionomycin in the presence of BD GolgiStop™ Protein Transport Inhibitor. The cells were permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution and stained with an 8-color interleukin panel [BD Horizon™ BV510 Mouse Anti-Human CD3 (clone UCHT1), BD Pharmingen™ PE-Cy™7 Mouse Anti-Human CD4 (clone SK3), BD Pharmingen™ APC Mouse Anti-Human CD8 (clone RPA-T8), BD Pharmingen™ FITC Mouse Anti-Human IFN γ (clone B27), BD Horizon™ BV421 Mouse Anti-Human IL-17A (clone N49-653), BD Pharmingen™ PE Mouse Anti-Human IL-2 (clone MQ1-17H12), BD Horizon™ BV786 Mouse Anti-Human IL-10 (clone JES3-9D7), and BD Horizon™ R718 Mouse Anti-Human IL-4 (clone MP4-25D2)]. The interleukin panel shown above is based on a CD4⁺ gate (A) or CD8⁺ gate (B), co-stained with BD Pharmingen™ FITC Mouse Anti-Human IFN γ , based on a lymphocyte (FSC/SSC) and CD3⁺ gate and analyzed on the BD FACSLyric™ Clinical System.

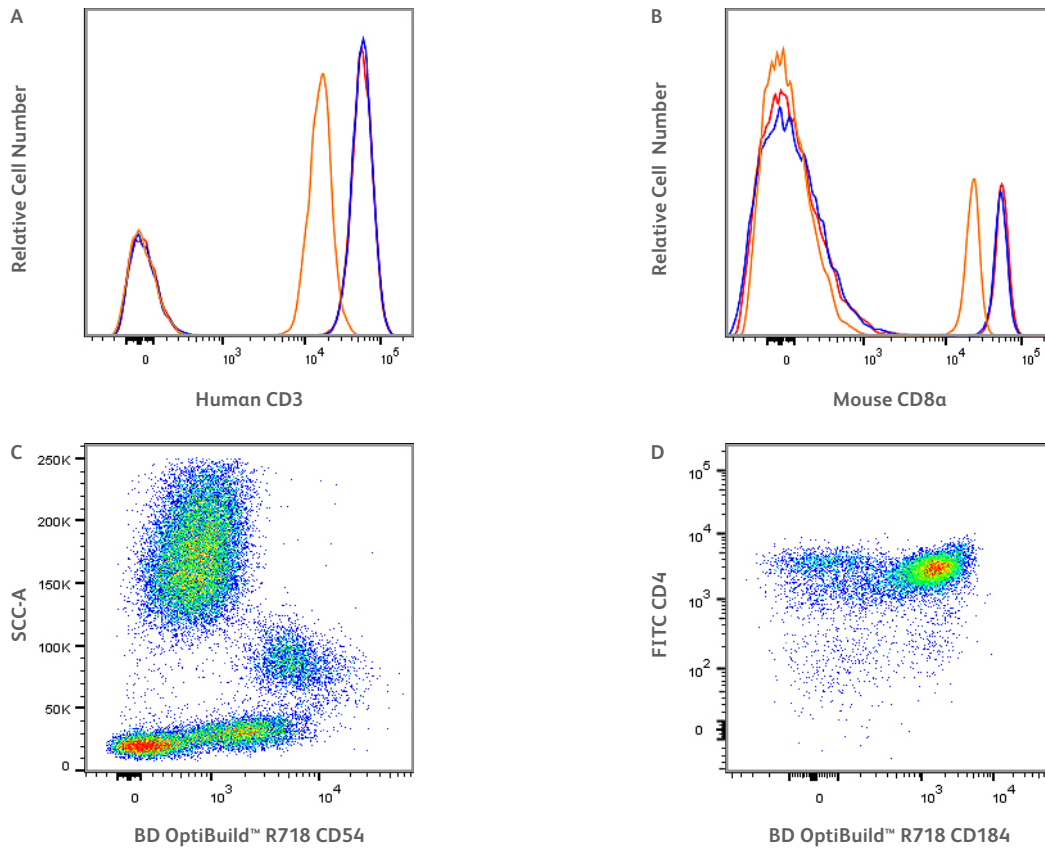


Figure 7. BD OptiBuild™ R718 Reagents and BD Horizon™ R718 Reagents offer superior resolution when compared to Alexa Fluor® 700.

Hundreds of additional R718 antibody combinations will be continuously introduced through the BD OptiBuild™ On-Demand Reagent program, providing researchers with increased flexibility for panel design. **A.** Comparison of human R718 and Alexa Fluor® 700 conjugates. Histogram overlays show BD Horizon™ R718 (red), BD OptiBuild™ R718 equivalent (blue), or BD Pharmingen™ Alexa Fluor® 700 (orange) conjugates of mouse anti-human CD3 (left, clone UCHT1). **B.** Histogram overlays show BD Horizon™ R718 (red), BD OptiBuild™ R718 equivalent (blue), or BD Pharmingen™ Alexa Fluor® 700 (orange) conjugates of rat anti-mouse CD8a (right, clone CD8a). Histograms were derived from gated events based on light scattering characteristics for intact splenocytes. **C.** Flow cytometric analysis of CD54 expression on human peripheral blood. Whole blood was stained with BD OptiBuild™ R718 Mouse Anti-Human CD54 Antibody (clone HA58). Erythrocytes were lysed with BD FACS™ Lysing Solution. The bivariate pseudocolor density plot showing the correlated expression of CD54 versus side-light scatter (SSC-A) signals was derived from gated events with the forward and side-light scatter characteristics. **D.** Multicolor flow cytometric analysis of mouse CD184 (CXCR4) expression on BALB/c thymocytes. BALB/c thymocytes were stained with FITC rat anti-mouse CD4 antibody (clone RM4-5) and BD OptiBuild™ R718 Rat Anti-Mouse CD184 Antibody (clone 2B11/CXCR4). The two color fluorescent dot plots were derived from gated events based on the light scattering characteristics for viable thymocytes. Flow cytometry was performed using a BD LSRFortessa™ X-20 Flow Cytometry System and FlowJo™ Software.

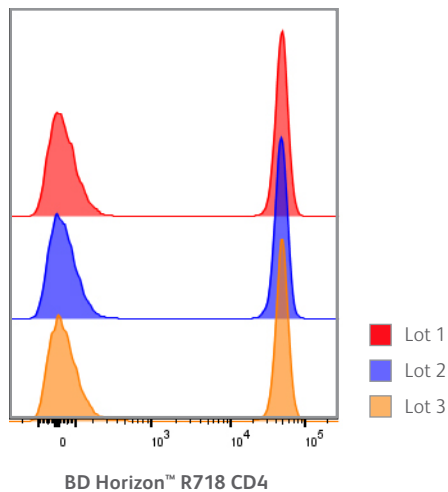


Figure 8. BD Horizon™ R718 Reagents are quality controlled for lot-to-lot consistency to reduce risk of data variability.

Three production lots of R718 dye were conjugated to Hu CD4 (clone SK3). The conjugates were analyzed side by side, results are shown as overlaid fluorescence histograms derived from a lymphocyte gate (Lot 1: Red line, Lot 2: Blue line, Lot 3: Orange line). BD employs stringent quality production standards to help ensure lot-to-lot consistency and reproducible results.

With one of the largest portfolios available for the 700 nm detector, you can now assign BD Horizon™ R718 Reagents to your low expression markers and assign BD's other bright dyes to help optimize panel performance.

Discover how BD Horizon™ R718 Reagents can help propel your research.

Featured Reagents*

| To view a complete list of products, including BD OptiBuild™ On-Demand Reagents, visit bdsciences.com/R718 For custom conjugations or bulk orders, email: BDB_Custom_Orders@bd.com or visit bdbiosciences.com/en-us/custom-reagents | | | | | |
|--|---------|-----------------|---------------|-----------|----------------|
| Reagent Specificity | Species | Clone | Isotype | Size | Catalog number |
| BCL-6 | Human | K112-91 | Mouse IgG1, κ | 100 Tests | 566979 |
| CD3 | Human | UCHT1 | Mouse IgG1, κ | 100 Tests | 566953 |
| | | | | 25 Tests | 566954 |
| CD4 | Human | SK3 | Mouse IgG1, κ | 100 Tests | 566930 |
| | | | | 25 Tests | 566931 |
| CD11c | Human | B-ly6 | Mouse IgG1, κ | 100 Tests | 566932 |
| | | | | 25 Tests | 566933 |
| CD19 | Human | SJ25C1 | Mouse IgG1, κ | 100 Tests | 566946 |
| | | | | 25 Tests | 566947 |
| CD279 (PD-1) | Human | EH12.1 | Mouse IgG1, κ | 100 Tests | 566974 |
| | | | | 25 Tests | 566975 |
| FoxP3 | Human | 259D/C7 | Ms IgG1 | 100 Tests | 566935 |
| | | | | 25 Tests | 566936 |
| IL-17A | Human | N49-653 | Mouse IgG1, κ | 100 Tests | 566938 |
| Stat5 (pY694) | Human | 47/Stat5(pY694) | Mouse IgG1, κ | 100 Tests | 566977 |
| | | | | 25 Tests | 566978 |
| CD107a | Mouse | 1D4B | Rat IgG2a, κ | 50 µg | 566986 |
| CD4 | Mouse | RM4-5 | Rat IgG2a, κ | 50 µg | 566939 |
| CD8a | Mouse | 53-6.7 | Rat IgG2a, κ | 50 µg | 566985 |
| CD13 | Mouse | R3-242 | Rat IgG1 | 50 µg | 566982 |
| Ly-6C | Mouse | AL-21 | Rat IgM, κ | 50 µg | 566987 |

* Reagents available in most countries. Ask your BD Representative for more information.

Class 1 Laser Product.

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