

# 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

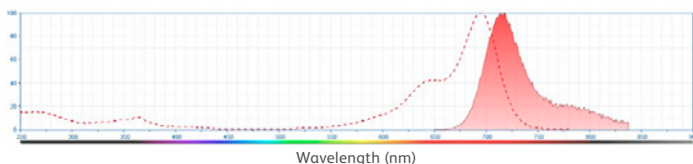



Figure 1. Excitation and emission spectra of R718 Reagents

R718 Specifications		
Ex <sub>max</sub>	Em <sub>max</sub>	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

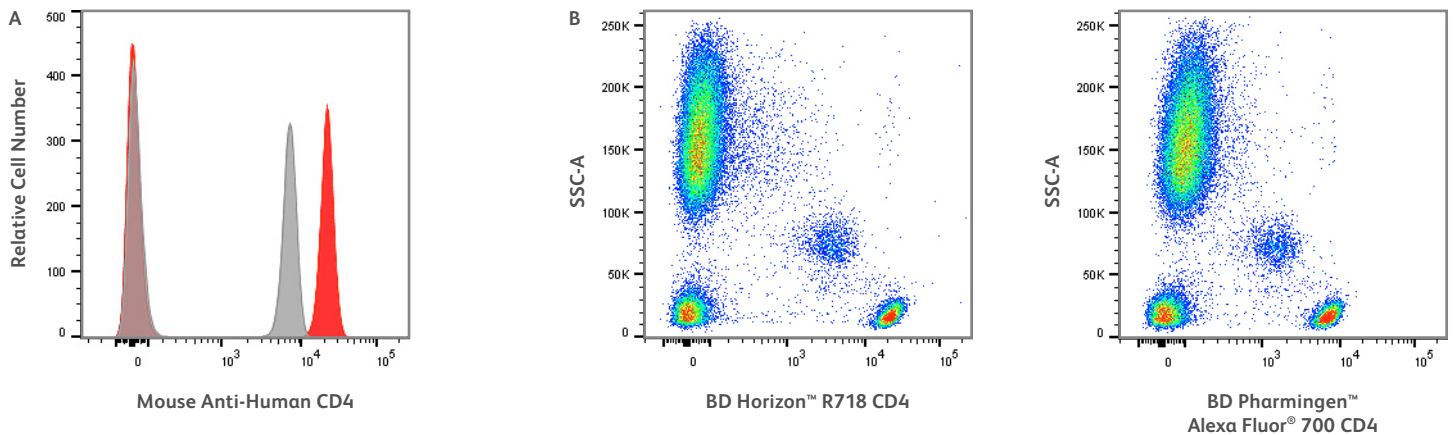
**Convenience:** Hundreds of reagents available through BD OptiBuild™ On-Demand Reagents

**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

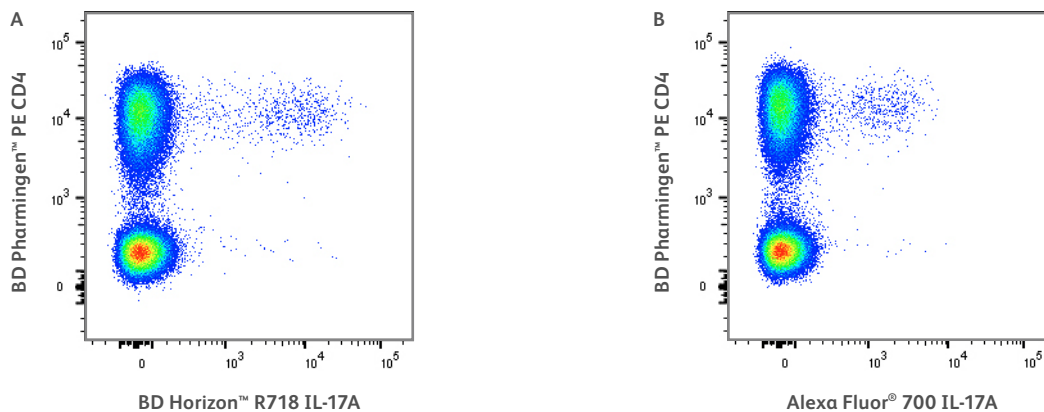
**Table 1. BD Horizon™ Red 718 Reagents have a higher stain index than Alexa Fluor® 700.**

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

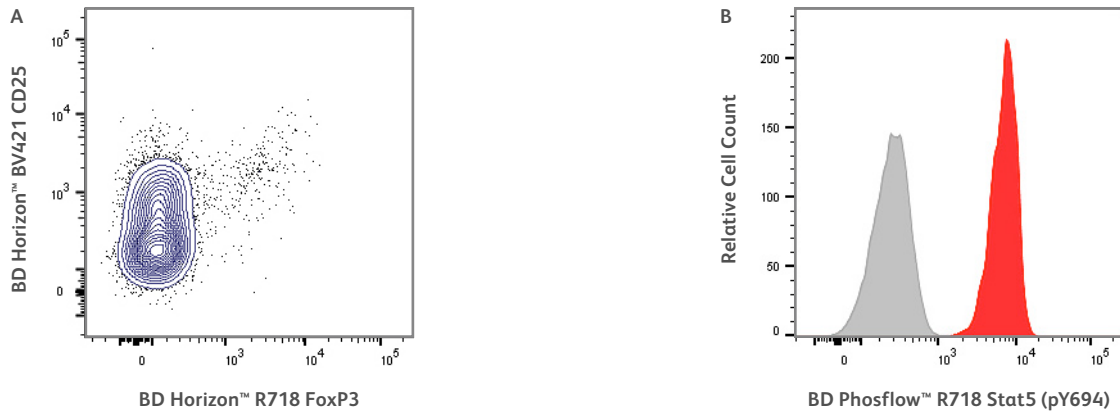


Figure 4. BD Horizon™ R718 Reagent intracellular staining of FoxP3 and Stat5.

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<sup>+</sup> gate.

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

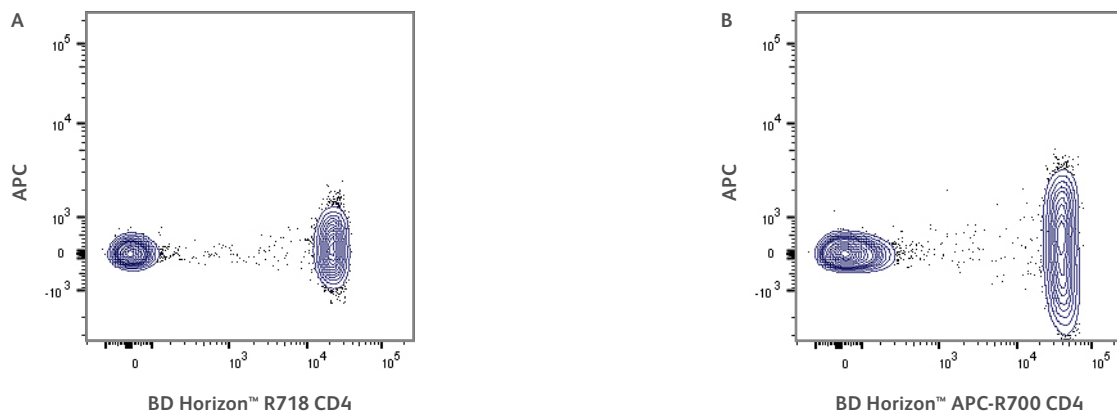
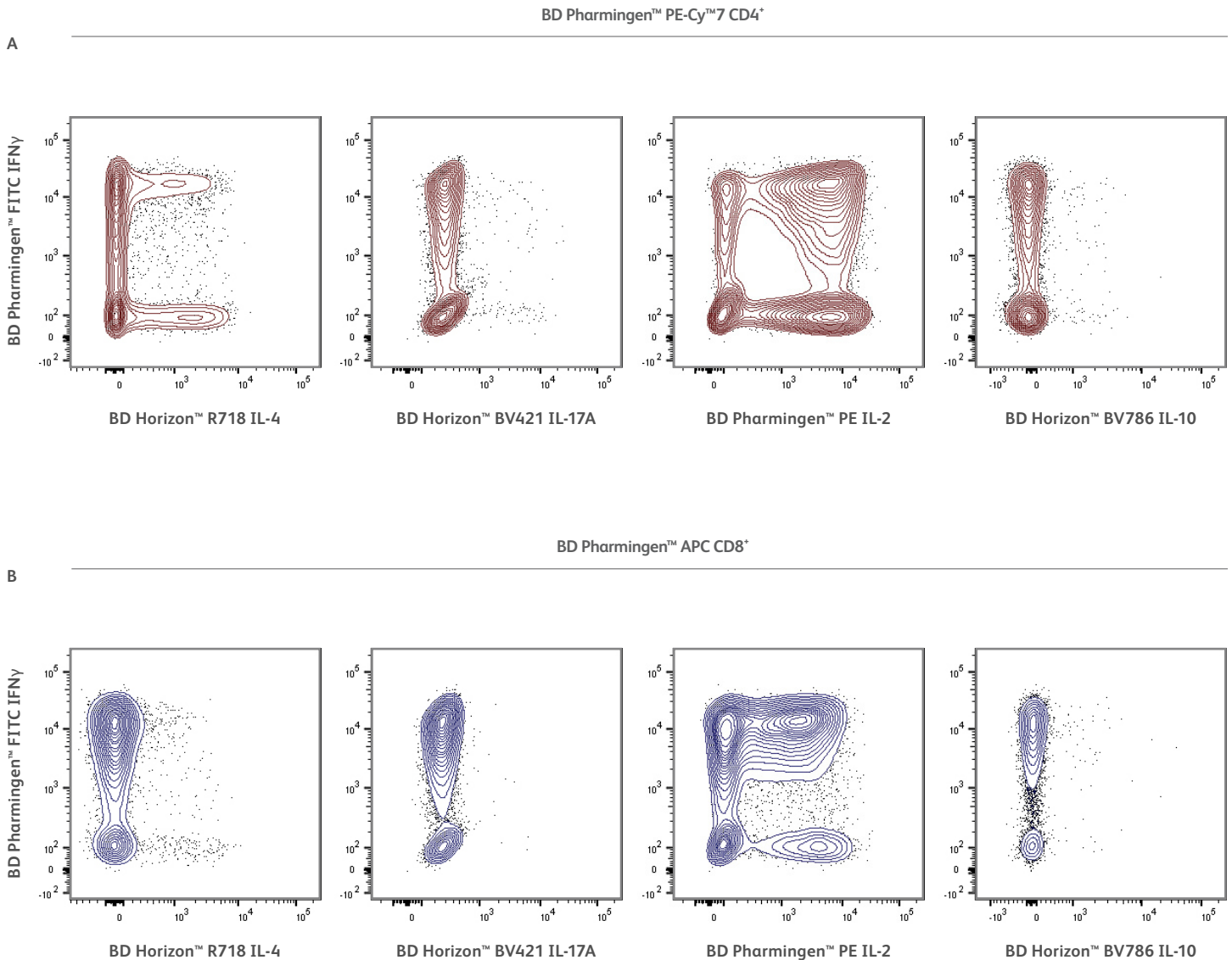


Figure 5. Relatively low spread was observed from BD Horizon™ R718 Reagents when compared to BD Horizon™ APC-R700 in the APC detector.

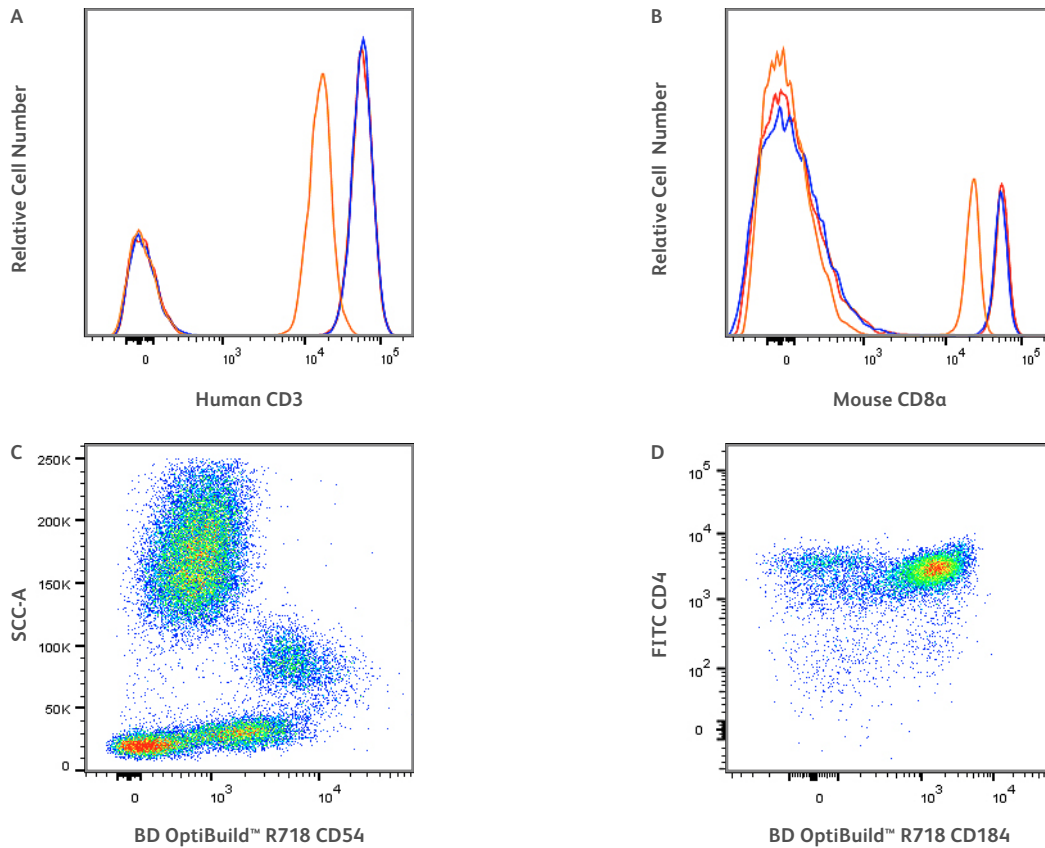
Lysed whole blood was stained with mouse anti-human CD4 (clone SK3) antibodies conjugated with (A) BD Horizon™ R718 and (B) BD Horizon™ APC-R700. Both reagents were analyzed on the same day on a BD LSRFortessa™ X-20 Cell Analyzer and analyzed with FlowJo™ Software.

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



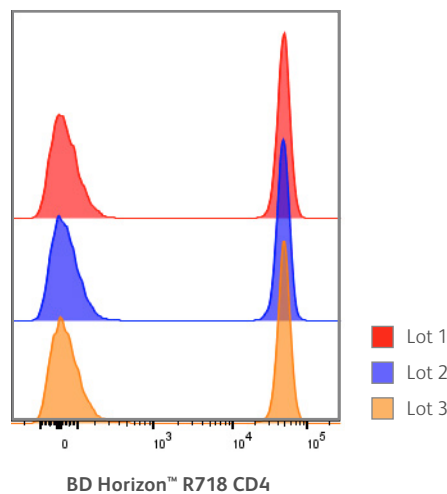
**Figure 6. BD Horizon™ R718 Reagents demonstrate excellent resolution of IL-4 as shown in left plots for both CD8<sup>+</sup> and CD4<sup>+</sup> T cells in a human T-cell cytokine response panel.**

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 $\gamma$  (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<sup>+</sup> gate (A) or CD8<sup>+</sup> gate (B), co-stained with BD Pharmingen™ FITC Mouse Anti-Human IFN $\gamma$ , based on a lymphocyte (FSC/SSC) and CD3<sup>+</sup> 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 CD8α (right, clone CD8α). 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 <a href="https://bdbiosciences.com/R718">bdbiosciences.com/R718</a> For custom conjugations or bulk orders, email: <a href="mailto:BDB_Custom_Orders@bd.com">BDB_Custom_Orders@bd.com</a> or visit <a href="https://bdbiosciences.com/en-us/custom-reagents">bdbiosciences.com/en-us/custom-reagents</a>					
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.

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

23-22633-00

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[bdbiosciences.com](http://bdbiosciences.com)

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