



BD Horizon RealBlue™ 744 Reagents

High-resolution fluorochrome to easily integrate into your higher parameter flow cytometry panels

BD Horizon RealBlue™ 744 (RB744) Reagents are part of a comprehensive family of laser-specific reagents. The RB744 fluorochrome is specially designed to produce less spillover, which improves panel resolution, enabling high-parameter experiments for spectral flow instruments.

RB744 is a bright fluorochrome well suited for low/medium-expression surface and intracellular markers and works well on the line of BD FACSymphony™ Analyzers and also on spectral flow cytometers.

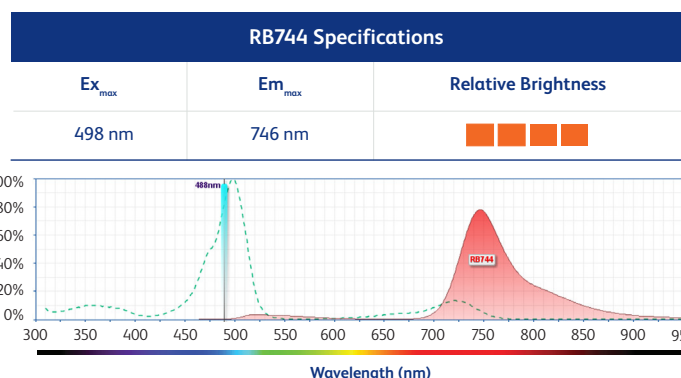


Figure 1. Excitation and emission spectra of the RB744 fluorochrome

The new laser-specific BD Horizon™ RB744 Fluorochrome can be used with low antigen–expression markers.

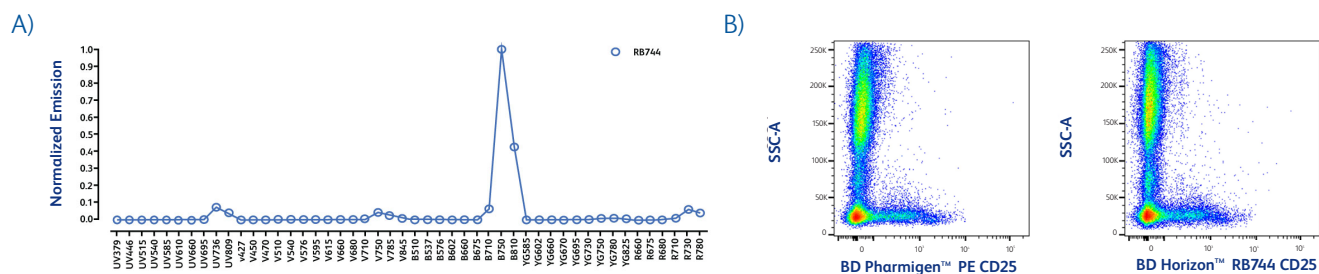


Figure 2. RB744 has minimal cross-laser excitation from the 561-nm yellow-green laser and can easily resolve low-expression markers

A) Normalized emission profile of RB744, demonstrating the low emission into UV, violet, yellow-green and red channels. B) Human whole blood was stained with PE (left) or BD Horizon™ RB744 Reagent (right) CD25 (2A3), co-stained with APC CD4 (SK3, data not shown), lysed with BD Pharm Lyse™ Lysing Buffer and acquired on a BD FACSsymphony™ A5 SE Cell Analyzer with compensation.

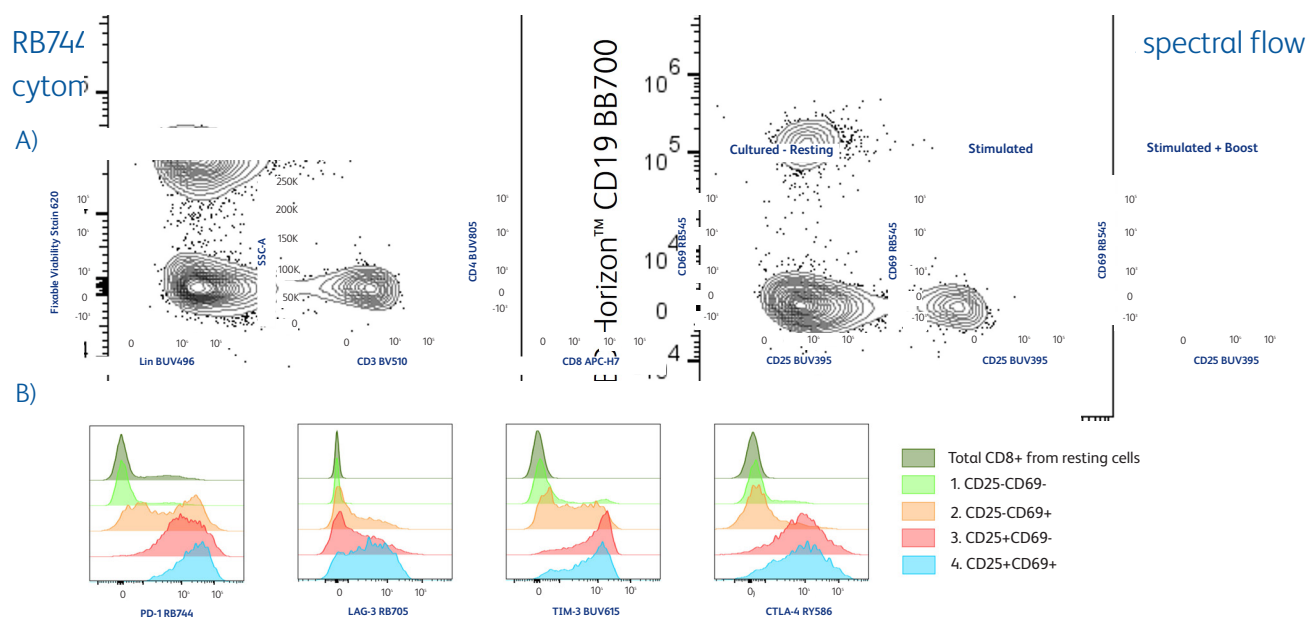


Figure 3. Expression of inhibitory markers on activated T cells as compared to resting T cells stained with a 17-color T cell panel containing RB744

Peripheral blood mononuclear cells were isolated and loaded with BD Horizon™ Violet Proliferation Dye 450 before stimulation with or without staphylococcal enterotoxin B (SEB, 1 µg/mL) and CD28 (1 µg/mL) for 3 days. Cells were then stained with BD Horizon™ Fixable Viability Stain 620 and antibodies against cell surface markers prior to fixing and permeabilizing with BD Cytofix/Cytoperm™ Fixation/Permeabilization Buffer. Stained cells were acquired on a BD FACSsymphony™ A5 SE Cell Analyzer and analyzed with FlowJo™ Software. A) Gating strategy for detection of T cell subsets after exclusion of doublets, dead cells and lineage-positive cells. CD8+ T cells were further evaluated for activation stage based on their expression of CD25 and CD69. B) Histogram overlays showing expression of inhibitory receptors on CD8+ T cell subsets from the Stimulated group. Total CD8+ T cells from the Unstimulated group (top, dark green).

BD flow cytometers are Class 1 Laser Products.

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