

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" Analysers and also on spectral flow cytometers.

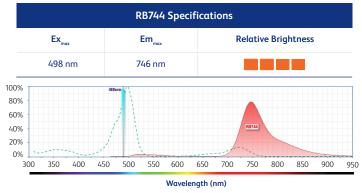


Figure 1. Excitation and emission spectra of the RB744 fluorochrome



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

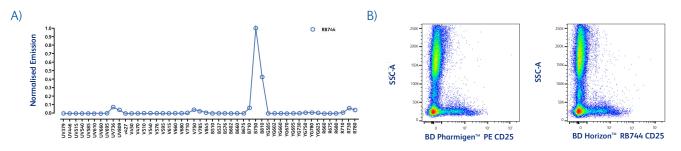


Figure 2. RB744 has minimal cross-laser excitation from the 561 nm yellow-green laser and can easily resolve low-expression markers A) Normalised emission profile of RB744, demonstrating the low emission into UV, violet, yellow-green and red channels. B) Human whole blood was

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 FACSymphony" A5 SE Cell Analyser with compensation.

RB744 easily detects the T cell inhibitory molecule CD279 (PD-1) upon cell activation in a spectral flow cytometry panel

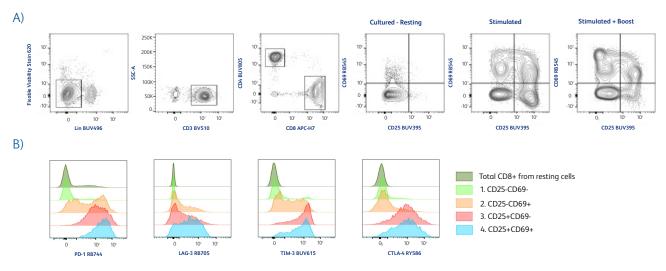


Figure 3. Expression of inhibitory markers on activated T cells as compared to resting T cells stained with a 17-colour T cell panel containing RB744

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 permeabilising with BD Cytofix/Cytoperm[®] Fixation/Permeabilisation Buffer. Stained cells were acquired on a BD FACSymphony[®] A5 SE Cell Analyser and analysed 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).



To request a sample or place an order, visit **bdbiosciences.com/real** or contact your local BD sales representative.

BD flow cytometers are Class 1 Laser Products. For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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