Technical Data Sheet

BB515 Mouse Anti-Human RANTES

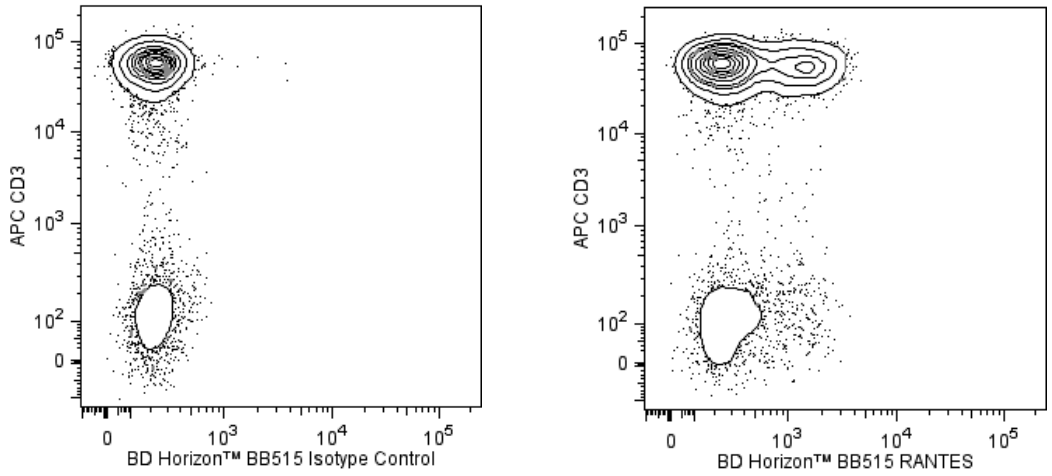
Product Information

Material Number:	**564752**
Alternate Name:	CCL5; chemokine (C-C motif) ligand 5; EoCP; SCYA5; SIS-delta; SISd; TCP228
Size:	50 Tests
Vol. per Test:	5 µl
Clone:	2D5
Immunogen:	Recombinant Human RANTES Protein
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 2D5 monoclonal antibody specifically binds to human Regulated upon Activation, Normal T cells Expressed and Secreted (RANTES). RANTES belongs to the C-C family of chemokines and is also known as CCL5. RANTES is produced by activated macrophages, CD8+ T cells, platelets, fibroblasts, epithelial cells and some tumor cells. RANTES is a chemoattractant and response modifier for a variety of cells including memory T cells, NK cells, dendritic cells, monocytes, eosinophils, basophils, and mast cells. RANTES binds to and transduces intracellular signals through several cell surface chemokine receptors including CCR1, CCR3, CCR4, and CCR5. RANTES can reportedly inhibit the interaction between certain HIV viruses and CCR5 and thus suppress viral infection in vitro.

The antibody was conjugated to BD Horizon BB515 which is part of the BD Horizon Brilliant™ Blue family of dyes. With an Ex Max near 490 nm and an Em Max near 515 nm, BD Horizon BB515 can be excited by the blue laser (488 nm) laser and detected with a 530/30 nm filter. This dye has been exclusively developed by BD Biosciences and is up to seven times brighter than FITC with less spillover into the PE channel. Due to similar excitation and emission properties, BB515, FITC, and Alexa Fluor® 488 cannot be used simultaneously. It is not recommended to use BB515 in cocktails that include Streptavidin conjugates as it may cause high background.



Two-color flow cytometric analysis of RANTES expression in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were cultured overnight in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) (Cat. No. 554724). The cells were harvested, washed with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), and fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655). The cells were then permeabilized and stained for 30 minutes at room temperature in BD Perm/Wash™ Buffer (Cat. No. 554723) with APC Mouse Anti-Human CD3 antibody (Cat. No. /561810/561811) and either BD Horizon™ BB515 Mouse IgG1 κ Isotype Control (Cat. No. 564416; Left Panel) or BD Horizon BB515 Mouse Anti-Human RANTES antibody (Cat. No. 564752/566043; Right Panel) using BD Biosciences Intracellular Cytokine Staining protocol. Two-color flow cytometric contour plots showing the correlated expression of RANTES (or Ig Isotype control staining) versus CD3 were derived from gated events with the forward and side light-scatter characteristics of intact stimulated lymphocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon™ BB515 under optimum conditions and unconjugated antibody was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

For optimal results, it is recommended to perform staining for 30-60 minutes at room temperature. Shorter staining times or staining performed at colder temperatures (4°C) may result in suboptimal signal. It is also recommended to perform 2 washes after staining with antibodies. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and CompBead to ensure that BD Comp beads are appropriate for your specific cellular application.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

For optimal results, it is recommended to perform 2 washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescence staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

Suggested Companion Products

Catalog Number	Name	Size	Clone
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
563794	Brilliant Stain Buffer	100 Tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 mL	(none)
554655	Fixation Buffer	100 mL	(none)
554723	Perm/Wash Buffer	100 mL	(none)
555335	APC Mouse Anti-Human CD3	100 Tests	UCHT1
561810	APC Mouse Anti-Human CD3	25 Tests	UCHT1
561811	APC Mouse Anti-Human CD3	500 Tests	UCHT1
554657	Stain Buffer (BSA)	500 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
566043	BB515 Mouse Anti-Human RANTES	25 Tests	2D5
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
6. Please refer to www.regdocs.bd.com to access safety data sheets (SDS).
7. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.

References

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128. (Methodology)

Sticherling M, Küpper M, Koltrowitz F, et al. Detection of the chemokine RANTES in cytokine-stimulated human dermal fibroblasts. *J Invest Dermatol.* 1995; 105(4):585-91. (Immunogen: ELISA, Immunohistochemistry, Western blot)