Technical Data Sheet

BB515 Rat Anti-Human CX3CR1

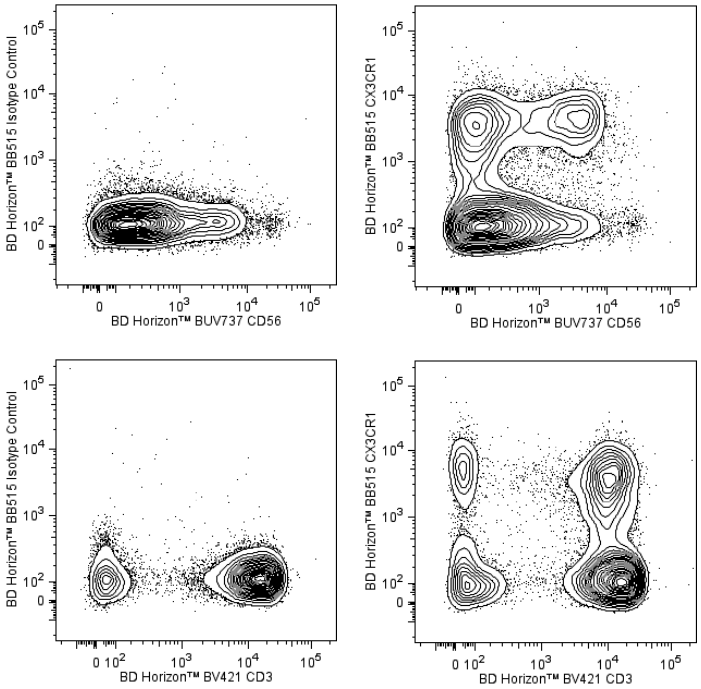
Product Information

Material Number:	**565902**
Alternate Name:	CCRL1; CMKBRL1; CMKDR1; GPR13; GPRV28; V28; Fractalkine Receptor
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	2A9-1
Immunogen:	Human CX3CR1 Recombinant Protein
Isotype:	Rat IgG2b, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 2A9-1 monoclonal antibody specifically binds to human CX3CR1, which is also known as chemokine (C-C) receptor-like 1 (CCRL1), Beta chemokine receptor-like 1 (CMK-BRL-1), G protein-coupled receptor 13 (GPR13), or GPRV28 (V28). CX3CR1 is a seven transmembrane G protein coupled receptor that is expressed by NK cells, T cells, and monocytes. The cellular expression of CX3CR1 is correlated with high levels of intracellular perforin and granzyme B. CX3CR1 serves as a receptor for fractalkine (CX3CL1). Fractalkine is a transmembrane chemokine of the CX3C family that is expressed on activated endothelial cells, neurons, and astrocytes. Interaction of CX3CR1 with fractalkine initiates cellular adhesive and chemotactic responses.

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.



Multicolor flow cytometric analysis of CX3CR1 expression on human peripheral blood lymphocytes. *Whole blood was treated with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899) to lyse erythrocytes. After washing, the leucocytes were stained with BD Horizon™ BUV737 Mouse Anti-Human CD56 antibody (Cat. No. 564447; Top Plots), BD Horizon™ BV421 Mouse Anti-Human CD3 antibody (Cat. No. 562426/562427; Bottom Plots), and either BD Horizon™ BB515 Rat IgG2b, κ Isotype Control (Cat. No. 564421; Left Plots) or BD Horizon BB515 Rat Anti-Human CX3CR1 antibody (Cat. No. 565902/565903; Right Plots). Two-color flow cytometric contour plots showing the correlated expression of CD56 or CD3 versus CX3CR1 (or Ig Isotype control staining), were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometric analysis was performed using a BD LSRFortessa™ Flow Cytometer System.*

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.

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Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

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
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564421	BB515 Rat IgG2b, κ Isotype Control	0.1 mg	R35-38
565903	BB515 Rat Anti-Human CX3CR1	100 Tests	2A9-1
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
555899	Lysing Buffer	100 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
564447	BUV737 Mouse Anti-Human CD56	100 Tests	NCAM16.2
562427	BV421 Mouse Anti-Human CD3	25 Tests	UCHT1
562426	BV421 Mouse Anti-Human CD3	100 Tests	UCHT1

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. 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.
6. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
7. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

Kobayashi T, Okamoto S, Iwakami Y, et al. Exclusive increase of CX3CR1+CD28-CD4+ T cells in inflammatory bowel disease and their recruitment as intraepithelial lymphocytes. *Inflamm Bowel Dis.* 2007; 13(7):837-46. (Biology)

Kondo Y, Kimura O, Tanaka Y, et al. Differential Expression of CX3CL1 in Hepatitis B Virus-Replicating Hepatoma Cells Can Affect the Migration Activity of CX3CR1+ Immune Cells. *J Virol.* 2015; 89(14):7016-27. (Biology)

Nanki T, Imai T, Nagasaka K, et al. Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. *Arthritis Rheum.* 2002; 46(11):2878-83. (Clone-specific: Flow cytometry)

Nishimura M, Umehara H, Nakayama T, et al. Dual functions of fractalkine/CX3C ligand 1 in trafficking of perforin+/granzyme B+ cytotoxic effector lymphocytes that are defined by CX3CR1 expression. *J Immunol.* 2002; 168(12):6173-80. (Immunogen: Flow cytometry)

Siwetz M, Sundl M, Kolb D, et al. Placental fractalkine mediates adhesion of THP-1 monocytes to villous trophoblast. *Histochem Cell Biol.* 2015; 143(6):565-74. (Biology)