Technical Data Sheet

BB515 Mouse Anti-Human CD62L

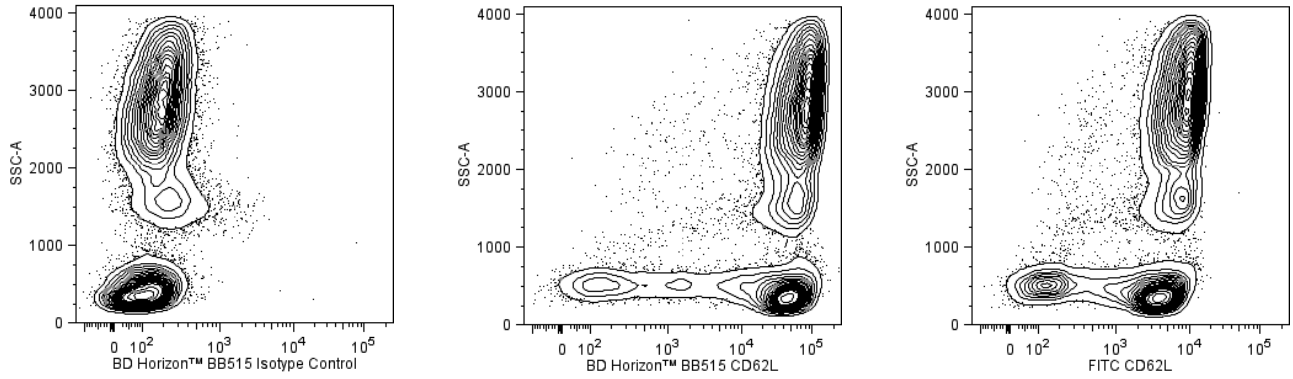
Product Information

Material Number:	**564743**
Alternate Name:	SELL; L-selectin; LSEL; LAM-1; LECAM-1; LEU8; LNHR; MEL-14; PLNHR; TQ-1
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	DREG-56
Immunogen:	Supernatant from PMA-activated Human Peripheral Blood Leukocytes
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	V S056
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The DREG-56 monoclonal antibody specifically binds to CD62L. CD62L is a 76-95 kDa glycoprotein that is also referred to as L-selectin or LECAM-1. CD62L is expressed on neutrophils, monocytes, T- and B-lymphocyte subsets and NK cells. The DREG-56 antibody recognizes the same antigen as LAM-1, and specifically inhibits >90% of binding of human lymphocytes to high endothelial venules (HEV) in frozen sections of peripheral, but not mucosal lymphoid tissue. It thus defines L-selectin as a human lymphocyte homing receptor for peripheral lymph node HEV.

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.



Multiparameter flow cytometric analysis of CD62L expression on human peripheral blood leucocyte populations - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. *Human whole blood was stained with either BD Horizon BB515 Mouse IgG1, κ Isotype Control (Cat. No. 564416; Left Panel), BD Horizon BB515 Mouse Anti-Human CD62L antibody (Cat. No. 564742/564743; Middle Panel), or FITC Mouse Anti-Human CD62L antibody (Cat. No. 555543/561914; Right Panel). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). Two-parameter flow cytometric contour plots showing the correlated expression of CD62L (or Ig Isotype control staining) versus side light-scatter (SSC-A) signals were derived from gated events with the forward and side light-scatter characteristics of intact leucocyte populations as indicated. Flow cytometric analysis was performed using a BD™ LSR II 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.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences

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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
563794	Brilliant Stain Buffer	100 Tests	(none)
564742	BB515 Mouse Anti-Human CD62L	100 Tests	DREG-56
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	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 http://regdocs.bd.com to access safety data sheets (SDS).
6. 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.
7. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

Kishimoto TK, Jutila MA, Butcher EC. Identification of a human peripheral lymph node homing receptor: a rapidly down-regulated adhesion molecule. *Proc Natl Acad Sci U S A.* 1990; 87(6):2244-2248. (Clone-specific: Inhibition)

Kishimoto TK, Warnock RA, Jutila MA, et al. Antibodies against human neutrophil LECAM-1 (LAM-1/Leu-8/DREG-56 antigen) and endothelial cell ELAM-1 inhibit a common CD18-independent adhesion pathway in vitro. *Blood.* 1991; 78(3):805-811. (Immunogen: Flow cytometry, Inhibition)

Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Clone-specific: Flow cytometry, Immunocytochemistry (cytospins), Immunohistochemistry)