Technical Data Sheet

BB515 Mouse Anti-Human CD11b

Product Information

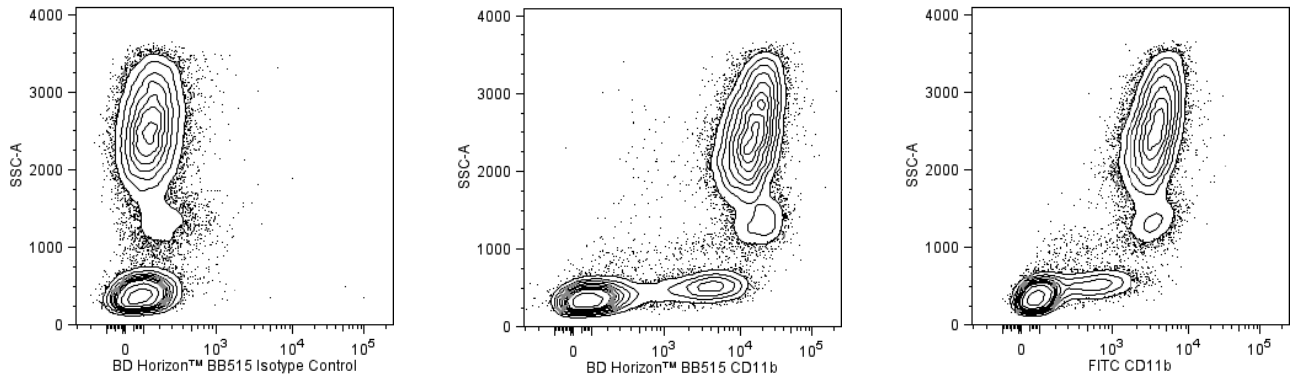
Material Number:	**564518**
Alternate Name:	MAC-1A; Mac-1; ITGAM; Integrin alpha M; CR3A; CR-3 alpha; Mo1; SLEB6
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	ICRF44 (also known as 44)
Immunogen:	Human monocytes
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	IV M047
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The ICRF44 monoclonal antibody specifically binds to CD11b, the 165-kDa adhesion glycoprotein that associates with the 95-kDa integrin β2 (CD18) to form the CD11b/CD18 complex, also known as Mac-1 or CR3. CD11b is a type I transmembrane glycoprotein that is encoded by *ITGAM* (Integrin alpha M). It is expressed on activated lymphocytes, monocytes, granulocytes, and a subset of NK cells. CD11b functions in cell-cell and cell-substrate interactions and is a receptor for iC3b, CD54 (ICAM-1), CD102 (ICAM-2) and CD50 (ICAM-3). This antibody significantly inhibits polymorphonuclear leukocyte aggregation in response to fMLP.

This clone also cross-reacts with granulocytes, a subset of peripheral blood lymphocytes and some monocytes of baboon, and both rhesus and cynomolgus macaque monkeys. The distribution on lymphocytes and granulocytes is similar to that observed with peripheral blood from normal human donors. There are fewer CD11b-positive monocytes present in the non-human primate blood than in normal human donor samples.

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 CD11b expression on human peripheral blood leucocytes - 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 CD11b antibody (Cat. No. 564517/564518; Middle Panel), or FITC Mouse Anti-Human CD11b antibody (Cat. No. 562793). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No.349202). Two-parameter flow cytometric contour plots showing the correlated expression of CD11b (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. 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 to the dye under optimum conditions and unconjugated antibody and free dye were removed.

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
563794	Brilliant Stain Buffer	100 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564517	BB515 Mouse Anti-Human CD11b	100 Tests	ICRF44
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 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. Species cross-reactivity detected in product development may not have been confirmed on every format and/or application.
6. Alexa Fluor® is a registered trademark of Life Technologies Corporation.
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.
8. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
9. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

David A, Kacher Y, Specks U, Aviram I. Interaction of proteinase 3 with CD11b/CD18 (beta2 integrin) on the cell membrane of human neutrophils. *J Leukoc Biol.* 2003; 74(4):551-557. (Biology)

Hogg N, Palmer DG, Revell PA. Mononuclear phagocytes of normal and rheumatoid synovial membrane identified by monoclonal antibodies. *Immunology.* 1985; 56(4):673-681. (Clone-specific: Immunohistochemistry)

Hogg N, Horton MA. Myeloid antigens: New and previously defined clusters. In: McMichael AJ. A.J. McMichael .. et al., ed. *Leucocyte typing III : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1987:576-602. (Clone-specific: Flow cytometry)

Zola H. *Leukocyte and stromal cell molecules : the CD markers.* Hoboken, N.J.: Wiley-Liss; 2007(Biology)