Technical Data Sheet

BB515 Mouse Anti-Human IgM

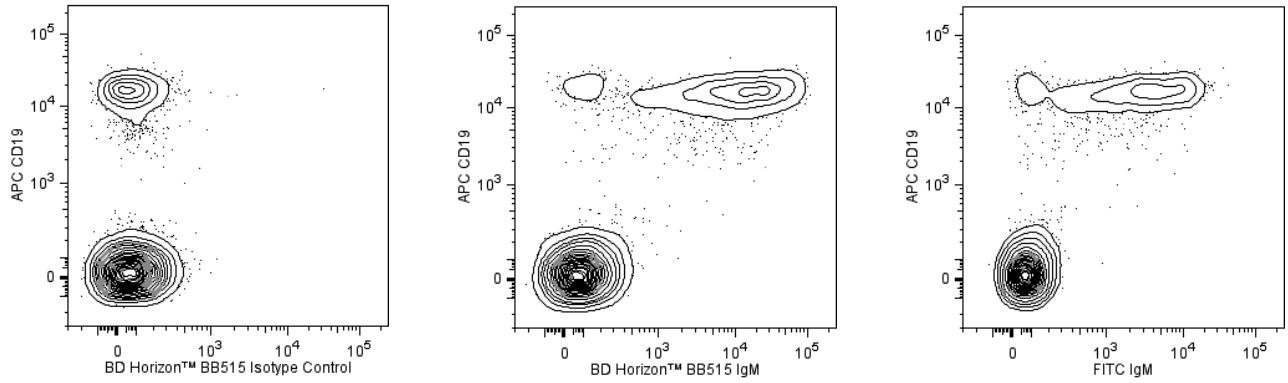
Product Information

Material Number:	**564622**
Alternate Name:	IGHM; MU; Ig mu chain C region; AGM1; VH
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	G20-127
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

IgM is an important component in the first line of defense against foreign pathogens, but may also play a role in autoimmune diseases. IgM monomers consist of two light and two heavy chains. Unlike the heavy chain of an IgG antibody which contains 3 constant Ig domains, the µ heavy chain of IgM contains 4 constant Ig domains. Five IgM monomers complex with a small polypeptide (J-chain) to form pentameric IgM that can be found in human plasma. In an immune response, the binding of IgM to a cell surface antigen enables C1q to activate interactions with downstream components in the classical complement pathway. Mature B lymphocytes express IgM. The G20-127 monoclonal antibody binds to the heavy chain of human IgM. The G20-127 antibody is not thought to react with other immunoglobulin heavy chain isotypes.

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 IgM expression on human peripheral blood lymphocytes - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. *Human peripheral blood mononuclear cells were washed and cultured in complete tissue culture medium overnight in order to minimize subsequent nonspecific immunofluorescent staining. The cells were harvested and stained with APC Mouse Anti-Human CD19 antibody (Cat. No. 555415/561742) and either BD Horizon BB515 Mouse IgG1, κ Isotype Control (Cat. No. 564416; Left Panel), BD Horizon BB515 Mouse Anti-Human IgM antibody (Cat. No. 564622; Middle Panel), or FITC Mouse Anti-Human IgM antibody (Cat. No. 555782/562029; Right Panel). Two-color flow cytometric contour plots showing the correlated expression of IgM (or Ig Isotype control staining) versus CD19 were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. 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
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
555415	APC Mouse Anti-Human CD19	100 Tests	HIB19
561742	APC Mouse Anti-Human CD19	25 Tests	HIB19
563794	Brilliant Stain Buffer	100 Tests	(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 www.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/pharmingen/protocols for technical protocols.

References

Chtanova T, Tangye SG, Newton R, et al. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *J Immunol.* 2004; 173(1):68-78. (Clone-specific: Flow cytometry)

Le Gallou S, Caron G, Delaloy C, Rossille D, Tarte K, Fest T. IL-2 requirement for human plasma cell generation: coupling differentiation and proliferation by enhancing MAPK-ERK signaling. *J Immunol.* 2012; 189(1):161-173. (Clone-specific: Flow cytometry)

Mei HE, Yoshida T, Sime W, et al. Blood-borne human plasma cells in steady state are derived from mucosal immune responses. *Blood.* 2009; 113(11):2461-2469. (Clone-specific: Flow cytometry, Immunofluorescence)

Widhopf GF, 2nd, Brinson DC, Kipps TJ, Tighe H. Transgenic expression of a human polyreactive Ig expressed in chronic lymphocytic leukemia generates memory-type B cells that respond to nonspecific immune activation. *J Immunol.* 2004; 172(4):2092-2099. (Clone-specific: Flow cytometry)

Zola H, Macardle PJ, Flego L, Webster J. The expression of sub-population markers on B cells: a re-evaluation using high-sensitivity fluorescence flow cytometry. *Dis Markers.* 1991; 9(2):103-118. (Biology: Cell differentiation, Dot Blot, Flow cytometry, Fluorescence activated cell sorting, In situ hybridization)