Technical Data Sheet

BUV737 Mouse Anti-Human CD16

Product Information

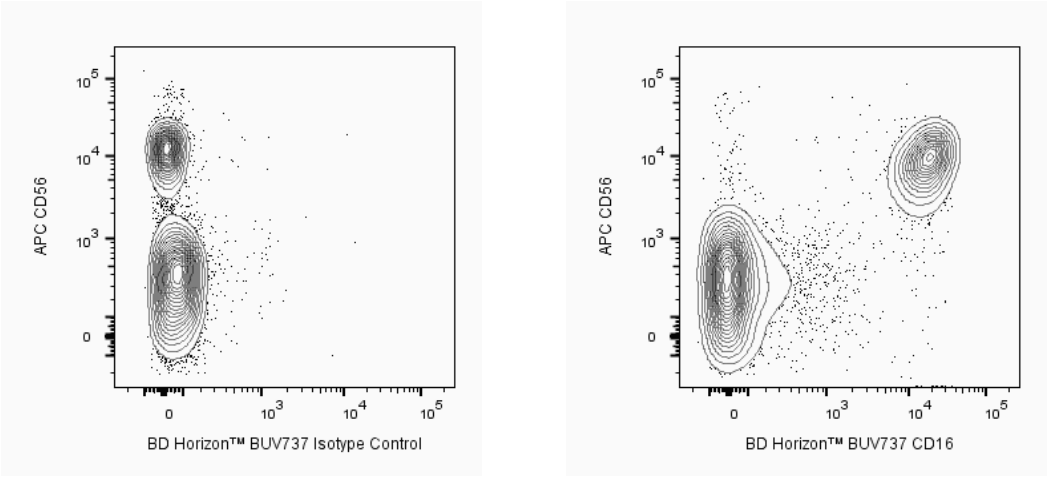
Material Number:	**612787**
Alternate Name:	CD16;CD16A;FCGR3A;FcγRIIIA;FcRIIIa;CD16B;FCGR3B;FcγRIIIB;FcRIIIb
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	3G8
Immunogen:	Human polymorphonuclear leukocytes
Isotype:	Mouse (BALB/c x DBA/2) IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	IV N409; V MR5, NK80
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 3G8 monoclonal antibody specifically recognizes CD16a and CD16b, low affinity receptors for the Fc region of IgG. CD16a is ~50-65 kDa type I transmembrane glycoprotein that is encoded by *FCGR3A* (Fc fragment of IgG receptor IIIa) which belongs to the immunoglobulin superfamily. CD16a is also known as Fc-gamma RIII-alpha (Fc-gamma RIIIa or FcγRIIIA) or FcRIIIa and is expressed on natural killer cells, activated monocytes, macrophages, γδ T cells, immature thymocytes, and mast cells. CD16a binds immune-complexed or aggregated IgG and associates with CD247/TCRζ in NK cells and FcεRIγ chains in phagocytes and mast cells to transduce intracellular signals. CD16a functions in antibody-dependent cellular cytotoxicity (ADCC) and other antibody-dependent responses including phagocytosis, cytokine production or mediator release. CD16b is a ~48 kDa glycophosyl-phosphatidylinositol (GPI)-linked form that is encoded by *FCGR3B* (Fc fragment of IgG receptor IIIb). CD16b is also known as Fc-gamma RIII-beta (Fc-gamma RIIIb or FcγRIIIB) or FcRIIIb and is expressed on neutrophils and activated eosinophils. The extracellular region of CD16b is highly homologous to CD16a. CD16b also serves as a receptor for the Fc region of IgG and can bind immune-complexed or aggregated IgG and may be involved in neutrophil adhesion.

The 3G8 antibody also crossreacts with a subset of peripheral blood lymphocytes and monocytes, but not granulocytes, of baboon, rhesus, and cynomolgus monkeys. Multicolor analysis reveals that the distribution on lymphocytes is similar to that found in human studies with the majority of CD16-positive lymphocytes being both CD3 and CD20 negative.

The antibody was conjugated to BD Horizon BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome with an Ex Max near 350 nm and an Em Max near 737 nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 nm filter. Due to the excitation of the acceptor dye by the red laser line, there may be significant spillover into red laser detectors with filters in the 700-720 nm range.



Two-color flow cytometric analysis of human CD16 expression on human peripheral blood cells. *Human peripheral blood cells were stained with APC Mouse Anti-Human CD56 antibody (Cat. No. 555518) and either BD Horizon™ BUV737 Mouse IgG1, κ Isotype Control (Cat. No. 612758; Left Plot) or BD Horizon BUV737 Mouse Anti-Human CD16 antibody (Cat. No. 612786/612787; Right Plot). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). The two-color flow cytometric contour plots showing the correlated expression of CD16 (or Ig Isotype control staining) versus CD56 were derived from gated events with the forward and side light-scatter characteristics of intact peripheral blood lymphocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ Cell Analyzer System and FlowJo™ software.*

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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 that minimize unconjugated dye and antibody.

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 BD 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 BD CompBead to ensure that BD CompBeads 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).

Note: When using high concentrations of antibody, background binding of this dye to erythroid cell subsets (mature erythrocytes and precursors) has been observed. For researchers studying these cell populations, or in cases where light scatter gating does not adequately exclude these cells from the analysis, this background may be an important factor to consider when selecting reagents for panel(s).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
612758	BUV737 Mouse IgG1, κ Isotype Control	50 µg	X40
612786	BUV737 Mouse Anti-Human CD16	100 Tests	3G8
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)

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. BD Horizon Brilliant Ultraviolet 737 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.
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

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Fleit HB, Wright SD, Unkeless JC. Human neutrophil Fc gamma receptor distribution and structure. *Proc Natl Acad Sci U S A.* 1982; 79(10):3275-3279. (Immunogen: Blocking, Immunoprecipitation, Inhibition, Radioimmunoassay)

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Wirthmueller U, Kurosaki T, Murakami MS, Ravetch JV. Signal transduction by Fc gamma RIII (CD16) is mediated through the gamma chain. *J Exp Med.* 1992; 175(5):1381-1390. (Clone-specific: Activation, Functional assay)
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