

Technical Data Sheet

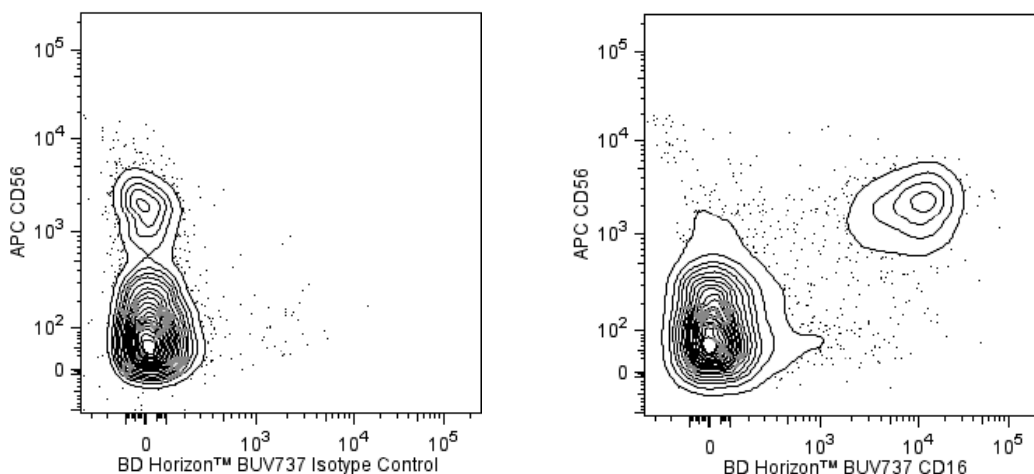
BUV737 Mouse Anti-Human CD16**Product Information**

Material Number:	564433
Alternate Name:	FcRIII; Fc-gamma RIII; FCG3; FCGR3; FCGRIII; FcγRIII; IGR3
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
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 3G8 monoclonal antibody specifically binds to the 50-65 kDa transmembrane form of the IgG Fc Receptor (FcγRIII), a human NK cell-associated antigen. CD16 is expressed on NK cells as well as macrophages and granulocytes. Reports indicate that CD16 plays a role in signal transduction and NK cell activation. The 3G8 antibody blocks the binding of soluble immune complexes to granulocytes. The 3G8 antibody is reported (Vossebeld *et al.*, 1997) to increase intracellular calcium levels in human neutrophils by interacting with both FcγRIIa and FcγRIIb molecules. This antibody has also been reported to induce homotypic neutrophil aggregation.

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. 564299; Left Panel) or BD Horizon BUV737 Mouse Anti-Human CD16 antibody (Cat. No. 564433/564434; Right Panel). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). The two-color flow cytometric dot plots show the correlated expression patterns of CD16 (or Ig Isotype control staining) versus CD56 for gated events with the forward and side light-scatter characteristics of intact peripheral blood 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 BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were 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 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)
564299	BUV737 Mouse IgG1, κ Isotype Control (to be replaced with 612758)	50 μ g	X40
564434	BUV737 Mouse Anti-Human CD16 (to be replaced with 612786 & 612787)	100 Tests	3G8
555518	APC Mouse Anti-Human CD56	100 Tests	B159
349202	BD FACST™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
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- μ l 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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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/pharming/en/protocols for technical protocols.

References

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