Technical Data Sheet

BV650 Mouse Anti-Human CD16

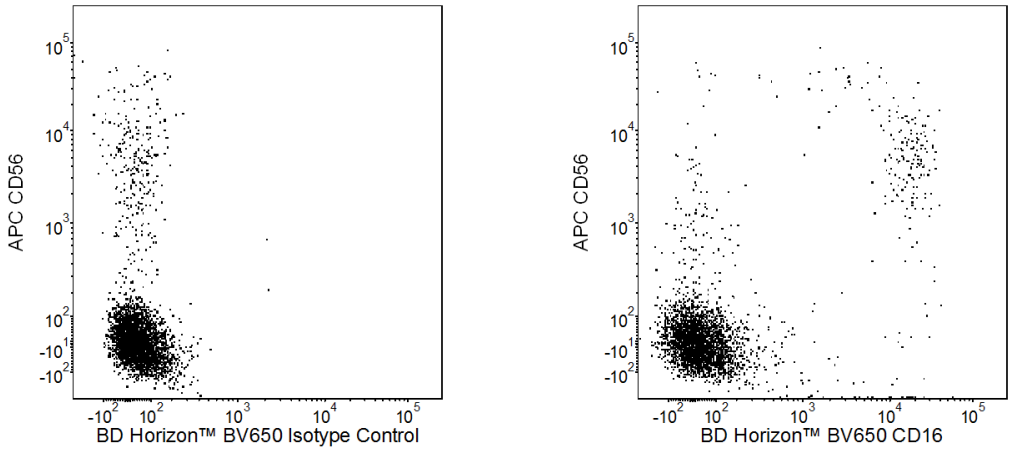
Product Information

Material Number:	**563692**
Alternate Name:	FcRIII; Fc-gamma RIII; FCG3; FCGR3; FCGRIII; FcγRIII; IGFR3
Size:	100 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 BSA and ≤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 FcγRIIIb molecules. This antibody has also been reported to induce homotypic neutrophil aggregation.

The antibody was conjugated to BD Horizon™ BV650 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. This dye is a tandem fluorochrome of BD Horizon™ BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 650-nm. BD Horizon™ BV650 can be excited by the violet laser and detected in a filter used to detect APC-like dyes (eg, 660/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there will be spillover into the APC and Alexa Fluor® 700 detectors. However, the spillover can be corrected through compensation as with any other dye combination.



Two-color flow cytometric analysis of human CD16 expression on human peripheral blood cells. *Human whole blood was stained with APC Mouse Anti-Human CD56 antibody (Cat. No. 555518) and either BD Horizon™ BV650 Mouse IgG1, κ Isotype Control (Cat. No. 563231; Left Panel) or BD Horizon™ BV650 Mouse Anti-Human CD16 antibody (Cat. No. 563691/563692; Right Panel). The erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). 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 viable 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™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

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Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563231	BV650 Mouse IgG1, k Isotype Control	50 µg	X40
563691	BV650 Mouse Anti-Human CD16	25 tests	3G8
555518	APC Mouse Anti-Human CD56	100 tests	B159
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Brilliant Violet™ 650 is a trademark of Sirigen.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

References

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: Bioassay, Immunoprecipitation, Inhibition, Radioimmunoassay)

Knapp W, Dörken B, Gilks WR, et al, ed. *Leucocyte Typing IV.* New York, NY: Oxford University Press; 1989:1-1182. (Clone-specific)

Perussia B, Starr S, Abraham S, Fanning V, Trinchieri G. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73.1. *J Immunol.* 1983; 130(5):2133-2141. (Biology)

Schmidt RE. Non-lineage/natural killer section report: new and previously defined clusters. In: Knapp W, Dörken B, Gilks WR, et al, ed. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:517-542. (Clone-specific)

Stroncek DF, Skubitz KM, Plachta LB, et al. Alloimmune neonatal neutropenia due to an antibody to the neutrophil Fc-gamma receptor III with maternal deficiency of CD16 antigen. *Blood.* 1991; 77(7):1572-1580. (Clone-specific: Immunofluorescence, Immunoprecipitation)

Vossebeld PJ, Homburg CH, Roos D, Verhoeven AJ. The anti-Fc gamma RIII mAb 3G8 induces neutrophil activation via a cooperative actin of Fc gamma RIIIb and Fc gamma RIIa. *Int J Biochem Cell Biol.* 1997; 29(3):465-473. (Clone-specific: Activation, Bioassay)

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, Bioassay, Immunoprecipitation)

Zola H, Swart B, Nicholson I, Voss E. *Leukocyte and Stromal Cell Molecules. The CD Markers.* Hoboken, New Jersey: John Wiley & Sons, Inc.; 2007:1-581. (Biology)

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