Technical Data Sheet

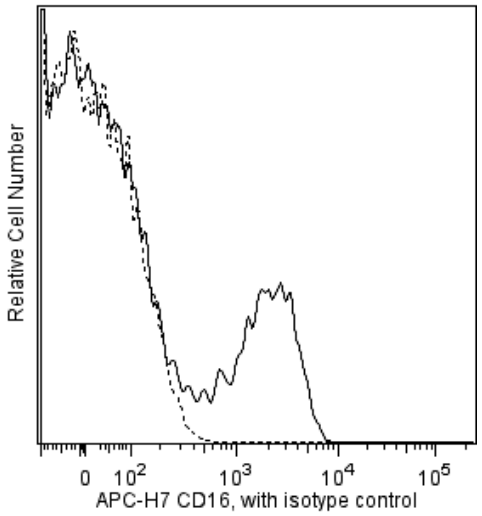
APC-H7 Mouse Anti-Human CD16

Product Information

Material Number:	**560195**
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 IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	IV N409
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, 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.



Flow cytometric analysis of APC-H7 anti-human CD16 on human lymphocytes. *Whole human blood was stained with APC-H7 Mouse anti-Human CD16 (Cat. No. 560195, solid line histogram) or with APC-H7 Mouse IgG1, κ Isotype Control (Cat. No. 560167; dashed line histogram). The erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry 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 APC-H7 under optimum conditions, and unconjugated antibody and APC-H7 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
560167	APC-H7 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554656	Stain Buffer (FBS)	500 ml	(none)
555899	Lysing Buffer	100 ml	(none)

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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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. 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.
5. BD APC-H7 is a tandem conjugate and an analog of APC-Cy7 with the same spectral properties. It has decreased intensity but it is engineered for greater stability and less spillover in the APC channel and consequently offers better performance than APC-Cy7. It has an absorption maximum of approximately 650 nm. When excited by light from a red laser, the APC fluorochrome can transfer energy to the cyanine dye, which then emits at a longer wavelength. The resulting fluorescent emission maximum is approximately 767 nm. BD recommends that a 750-nm longpass filter be used along with a red-sensitive detector such as the Hamamatsu R3896 PMT. As with APC-Cy7 special filters are required when using APC-H7 in conjunction with APC.
   Note: Although our APC-H7 products demonstrate higher lot-to lot consistency than other APC tandem conjugate products, and every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-H7 conjugate.
   Note: Cy is a trademark of Amersham Biosciences Limited.
6. Although BD APC-H7 is engineered to minimize spillover to the APC channel and is more stable and less affected by light, temperature, and formaldehyde-based fixatives, compared to other APC-cyanine tandem dyes, it is still good practice to minimize as much as possible, any light, temperature and fixative exposure when working with all fluorescent conjugates.
7. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
8. Cy is a trademark of Amersham Biosciences Limited.
9. An isotype control should be used at the same concentration as the antibody of interest.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Barclay NA, Brown MH, Birkeland ML, et al, ed. *The Leukocyte Antigen FactsBook.* San Diego, CA: Academic Press; 1997. (Biology)

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. (Biology)

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

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)

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. (Biology)

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