Technical Data Sheet

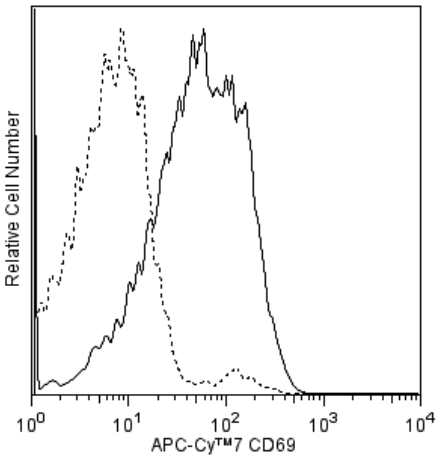
APC-Cy™7 Hamster Anti-Mouse CD69

Product Information

Material Number:	**561240**
Alternate Name:	VEA; Very Early Activation Antigen; AIM; Activation Induced Molecule
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	H1.2F3
Immunogen:	Mouse Dendritic Epidermal T Cell Line Y245
Isotype:	Armenian Hamster IgG1, λ3
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The H1.2F3 monoclonal antibody specifically binds to CD69 (Very Early Activation antigen), an 85 kDa disulfide-linked homodimer of differentially glycosylated subunits. CD69 is a C-type lectin, most closely related to the NKR-P1 and Ly-49 NK cell-activation molecules. Its expression is rapidly induced upon activation of lymphocytes (T, B, NK, and NK-T cells), neutrophils, and macrophages. CD69 is expressed also on thymocytes that are undergoing positive selection; its role in that process is unclear. H1.2F3 mAb augments PMA-induced T-cell stimulation and IFN-γ-induced macrophage stimulation. IL-2-activated NK cells express CD69, and H1.2F3 mAb induces redirected lysis of FcR-bearing target cells by NK cells.



Flow cytometric analysis of CD69 expression on stimulated mouse splenocytes. *BALB/c splenocytes were stimulated for 5 hours at 37°C with 10 ng/mL Phorbol 12-Myristate 13-Acetate (PMA; Sigma-Aldrich Cat. No. P-8139) and stained either with an APC-Cy™7 Hamster IgG1, λ1 Isotype Control (Cat. No.561206; dashed line histogram) or with the APC-Cy™7 Hamster Anti-Mouse CD69 antibody (Cat. No. 561240; solid line histogram). Histograms were derived from gated events with the forward and side light-scatter characteristics of viable cells. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with APC-Cy7 under optimum conditions, and unconjugated antibody and free APC-Cy7 were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
561206	APC-Cy™7 Hamster IgG1, λ1 Isotype Control	0.1 mg	G235-2356
554656	Stain Buffer (FBS)	500 ml	(none)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
5. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7™, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although 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-Cy7 conjugate.
6. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
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. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
10. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
11. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
12. This conjugated product is sold under license to the following patent: US Patent No. 5,714,386.

References

Bendelac A, Matzinger P, Seder RA, Paul WE, Schwartz RH. Activation events during thymic selection. *J Exp Med.* 1992; 175(3):731-742. (Biology)

Brandle D, Muller S, Muller C, Hengartner H, Pircher H. Regulation of RAG-1 and CD69 expression in the thymus during positive and negative selection. *Eur J Immunol.* 1994; 24(1):145-151. (Biology)

Gabor MJ, Godfrey DI, Scollay R. Recent thymic emigrants are distinct from most medullary thymocytes. *Eur J Immunol.* 1997; 27(8):2010-2050. (Biology)

Karlhofer FM, Yokoyama WM. Stimulation of murine natural killer (NK) cells by a monoclonal antibody specific for the NK1.1 antigen. IL-2-activated NK cells possess additional specific stimulation pathways. *J Immunol.* 1991; 146(10):3662-3673. (Biology)

Keefe R, Dave V, Allman D, Wiest D, Kappes DJ. Regulation of lineage commitment distinct from positive selection. *Science.* 1999; 286(5442):1149-1153. (Biology)

Lauzurica P, Sancho D, Torres M, et al. Phenotypic and functional characteristics of hematopoietic cell lineages in CD69-deficient mice. *Blood.* 2000; 95(7):2312-2320. (Biology)

Marzio R, Jirillo E, Ransijn A, Mauel J, Corradin SB. Expression and function of the early activation antigen CD69 in murine macrophages. *J Leukoc Biol.* 1997; 62(3):349-355. (Biology)

Merkenschlager M, Graf D, Lovatt M, Bommhardt U, Zamoyska R, Fisher AG. How many thymocytes audition for selection. *J Exp Med.* 1997; 186(7):1149-1158. (Biology)

Nishimura T, Kitamura H, Iwakabe K, et al. The interface between innate and acquired immunity: glycolipid antigen presentation by CD1d-expressing dendritic cells to NKT cells induces the differentiation of antigen-specific cytotoxic T lymphocytes. *Int Immunol.* 2000; 12(7):987-994. (Biology)

Punt JA, Suzuki H, Granger LG, Sharrow SO, Singer A. Lineage commitment in the thymus: only the most differentiated (TCRhibcl-2hi) subset of CD4+CD8+ thymocytes has selectively terminated CD4 or CD8 synthesis. *J Exp Med.* 1996; 184(6):2091-2099. (Biology)

Shapiro HM. *Practical Flow Cytometry, 3rd Edition.* New York: Wiley-Liss, Inc; 1995:280-281. (Methodology: Flow cytometry)

Sobel ES, Yokoyama WM, Shevach EM, Eisenberg RA, Cohen PL. Aberrant expression of the very early activation antigen on MRL/Mp-lpr/lpr lymphocytes. *J Immunol.* 1993; 150(2):673-682. (Biology)

Wilkinson RW, Anderson G, Owen JJ, Jenkinson EJ. Positive selection of thymocytes involves sustained interactions with the thymic microenvironment. *J Immunol.* 1995; 155(11):5234-5240. (Biology)

Yokoyama WM, Koning F, Kehn PJ, et al. Characterization of a cell surface-expressed disulfide-linked dimer involved in murine T cell activation. *J Immunol.* 1988; 141(2):369-376. (Immunogen: Flow cytometry)

Yokoyama WM, Maxfield SR, Shevach EM. Very early (VEA) and very late (VLA) activation antigens have distinct functions in T lymphocyte activation. *Immunol Rev.* 1989; 109:153-176. (Biology)

Ziegler SF, Levin SD, Johnson L, et al. The mouse CD69 gene. Structure, expression, and mapping to the NK gene complex. *J Immunol.* 1994; 152(3):1228-1236. (Biology)

Ziegler SF, Ramsdell F, Alderson MR. The activation antigen CD69. *Stem Cells.* 1994; 12(5):456-465. (Biology)