Technical Data Sheet

PerCP-Cy™5.5 Hamster Anti-Mouse CD69

Product Information

Material Number:	**561931**
Alternate Name:	VEA; Very Early Activation Antigen; AIM; Activation Induced Molecule
Size:	25 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 ≤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.

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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

PerCP has been reported to undergo significant photobleaching, the magnitude of which increases as laser power is increased or beam focus is narrowed. For tandem conjugates incorporating PerCP (e.g., PerCP-Cy5.5), the excitation and emission properties of PerCP and the kinetics of energy exchange between the fluorochromes of the tandem dye may limit their effectiveness on high-speed and/or sorting flow cytometers. Therefore, for third color flow-cytometric analysis using ≥ 25-mW laser power, we recommend PE-Cy7-conjugated reagents (Cat. No. 552879).

It is recommended that a 712/20-nm band-pass filter be used with stream-in-air instruments such as the BD FACStar™ and BD FACSVantage™ flow cytometry systems.

Suggested Companion Products

Catalog Number	Name	Size	Clone
552879	PE-Cy™7 Hamster Anti-Mouse CD69	0.1 mg	H1.2F3
550763	PerCP-Cy™5.5 Hamster IgG1, κ Isotype Control	0.1 mg	A19-3
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://www.bdbiosciences.com/pharmingen/hamster_chart_11x17.pdf.
5. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.

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6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
7. 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.
8. 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.
9. 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.
10. 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.

References

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