Technical Data Sheet

BV605 Hamster Anti-Mouse CD69

Product Information

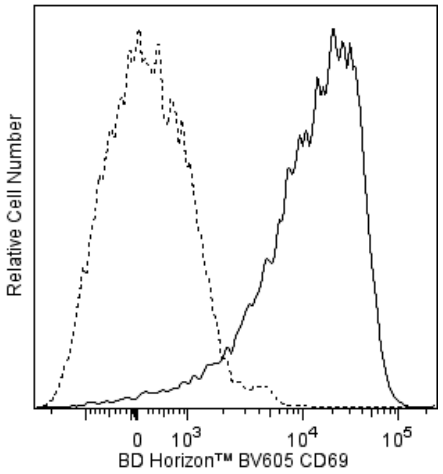
Material Number:	**563290**
Alternate Name:	VEA; Very Early Activation Antigen; AIM; Activation Induced Molecule
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	H1.2F3
Immunogen:	Mouse Dendritic Epidermal T Cell Line Y245
Isotype:	Armenian Hamster IgG1, λ3
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA 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.

This antibody is conjugated to BD Horizon BV605 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max of 602-nm, BD Horizon BV605 can be excited by a violet laser and detected with a standard 610/20-nm filter set. BD Horizon BV605 is a tandem fluorochrome of BD Horizon BV421 and an acceptor dye with an Em max at 605-nm. Due to the excitation of the acceptor dye by the green (532 nm) and yellow-green (561 nm) lasers, there will be significant spillover into the PE and BD Horizon PE-CF594 detectors off the green or yellow-green lasers. BD Horizon BV605 conjugates are very bright, often exhibiting brightness equivalent to PE conjugates and can be used as a third color off of the violet laser.

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).



Flow cytometric analysis of CD69 expression on stimulated mouse splenocytes. *C57BL/6 mouse splenic leucocytes were stimulated for 5 hours at 37°C with Phorbol 12-Myristate 13-Acetate (PMA, 10 ng/mL; Sigma-Aldrich, Cat. No. P-8139). The stimulated cells were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with either BD Horizon™ BV605 Hamster IgG1, λ1 Isotype Control (Cat. No. 563054; dashed line histogram) or BD Horizon™ BV605 Hamster Anti-Mouse CD69 antibody (Cat. No. 563290; solid line histogram). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable stimulated leucocytes. 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™ BV605 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV605 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)
563054	BV605 Hamster IgG1, λ1 Isotype Control	50 µg	G235-2356
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
555899	Lysing Buffer	100 mL	(none)
563794	Brilliant Stain Buffer	5 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. 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.
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. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from BD Horizon™ BV421 may be observed. Therefore, we recommend that individual compensation controls be performed for every BD Horizon™ BV605 conjugate.
9. 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/documents/hamster_chart_11x17.pdf.
10. CF™ is a trademark of Biotium, Inc.

References

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