Technical Data Sheet

PE-CF594 Mouse Anti-Human CD69

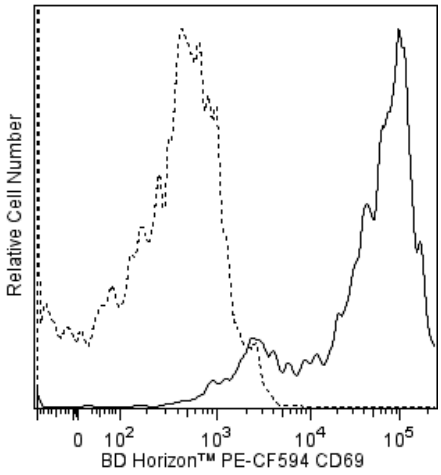
Product Information

Material Number:	**562645**
Alternate Name:	AIM; CLEC2C; EA1; GP32/28; Leu23; MLR-3; VEA; BL-AC/P26
Size:	50 tests
Vol. per Test:	5 µl
Clone:	FN50
Isotype:	Mouse IgG1, κ
Reactivity:	Human QC Testing: Rhesus, Cynomolgus, Baboon
Workshop:	IV A091
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The FN50 monoclonal antibody specifically binds to human CD69. CD69 is also known as activation-induced molecule (AIM), early activation antigen (EA-1), very early activation antigen (VEA), C-type lectin domain family 2 member C (CLEC2C), MLR-3, GP32/28 and Leu-23. CD69 is a transmembrane type II homodimer receptor. CD69 is comprised of disulfide-linked, differentially glycosylated core protein subunits that are approximately 28 and 34 kDa in size. Each subunit contains a C-type lectin domain. CD69 is expressed on activated T, B, and natural killer (NK) lymphocytes, thymocytes, neutrophils, eosinophils and platelets. In normal peripheral blood, a small and variable percentage of lymphocytes typically express detectable membrane CD69 antigen. Upon activation, CD69 antigen expression increases on lymphocytes. Peak CD69 expression generally occurs within 18 hours of activation, preceding the appearance of HLA-DR, IL-2Rα (CD25) and transferrin receptor (CD71). CD69 is highly expressed on the bright CD3+ subset of thymocytes. FN50 monoclonal antibody labels NK cells and most lymphocytes of the follicular mantle and perifollicular/interfollicular zone as well as germinal center T cells of lymph nodes and tonsils. Studies indicate that CD69 serves as a signaling receptor in the activation of a variety of cell types.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Flow cytometric analysis of CD69 expression on stimulated Rhesus peripheral blood mononuclear cells. *Rhesus peripheral blood mononuclear cells were stimulated overnight with 20 ng/mL Phorbol 12-Myristate 13-Acetate (PMA; Sigma-Aldrich Cat. No. P-8139) and 250 ng/mL calcium ionophore A23187 (Sigma-Aldrich Cat. No. C-9275). Cells were then stained with either a BD Horizon™ PE-CF594 Mouse IgG1, κ Isotype Control (Cat. No. 562292; dashed line histogram) or with the BD Horizon™ PE-CF594 Mouse Anti-Human CD69 antibody (Cat. No. 562645; solid line histogram). 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 BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

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Suggested Companion Products

Catalog Number	Name	Size	Clone
562292	PE-CF594 Mouse IgG1, κ Isotype Control	0.1 mg	X40
554656	Stain Buffer (FBS)	500 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. 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. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. CF™ is a trademark of Biotium, Inc.
10. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
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12. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.

References

Knapp W, Dorken B, Rieber EP, et al, ed. *Leucocyte Typing IV*. New York: Oxford University Press; 1989:1-1208. (Clone-specific)
Schlossman S, Boumell L, et al, ed. *Leucocyte Typing V*. New York: Oxford University Press; 1995. (Biology)
Zola H, Swart B, Nicholson I, Voss E. *CD69. In: Leukocyte and Stromal Cell Molecules. The CD Markers.* Hoboken, New Jersey: John Wiley & Sons, Inc.; 2007:161. (Biology)

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