Technical Data Sheet

PE-Cy™5 Mouse Anti-Human CD69

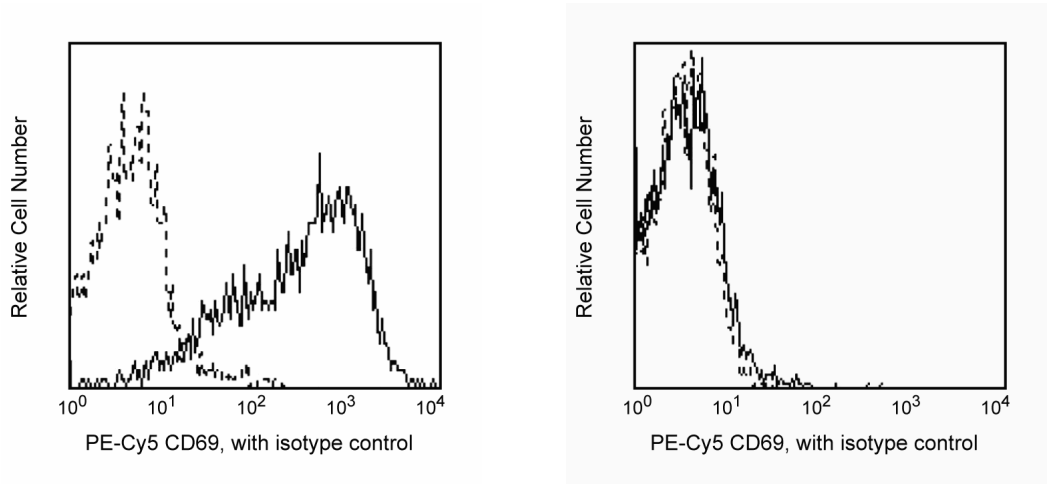
Product Information

Material Number:	**555532**
Alternate Name:	AIM; CLEC2C; EA1; GP32/28; Leu23; MLR-3; VEA; BL-AC/P26
Size:	100 Tests
Vol. per Test:	20 µl
Clone:	FN50 (also known as FN 50)
Immunogen:	Anti-µ stimulated human B lymphocytes
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	IV A91 (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.

Clone FN50 reacts with the human form of the 28/34 kDa dimeric glycoprotein expressed early during activation of lymphocytes, monocytes and platelets. It also cross-reacts with a subset of peripheral blood mononuclear cells (lymphocytes and monocytes) of rhesus and cynomolgus macaque monkeys. The distribution on lymphocytes is similar to that observed with human peripheral blood lymphocytes with the majority of the cells demonstrating an increase in FN50 positivity following overnight incubation with phorbol myristate acetate (PMA).



Flow cytometric analysis of CD69 expressed on peripheral blood mononuclear cells. *Human PBMC were stimulated for 24 hours with phytohemagglutinin (Left Panel) or unstimulated (Right Panel). Cells were then stained with either a PE-Cy™5 Mouse IgG1, κ Isotype control (Cat. No. 555750; dashed line histogram) or with the PE-Cy™5 Mouse Anti-Human CD69 antibody (Cat. No. 555532; solid line histogram). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes.*

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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 PE-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
555750	PE-Cy™5 Mouse IgG1 κ Isotype Control	100 Tests	MOPC-21
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	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. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5™.
7. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
8. Cy is a trademark of GE Healthcare.
9. 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.
10. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.
11. PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc Block™ (the CD72c complex has been implicated for PE-Cy5 binding in these strains). Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.
12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Knapp W. W. Knapp .. et al., ed. *Leucocyte typing IV : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1989:1-1182. (Clone-specific)
Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Biology)