Technical Data Sheet

BUV737 Mouse Anti-Human CD69

Product Information

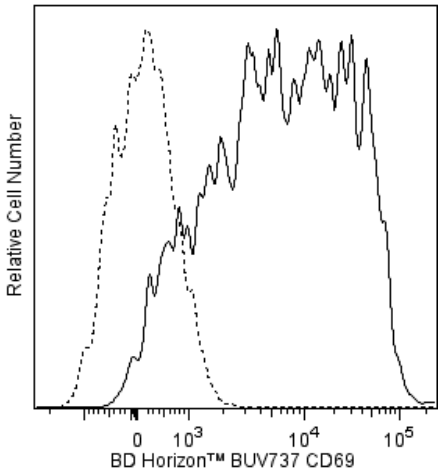
Material Number:	**564439**
Alternate Name:	AIM; CLEC2C; EA1; GP32/28; Leu23; MLR-3; VEA; BL-AC/P26
Size:	50 Tests
Vol. per Test:	5 µl
Clone:	FN50 (also known as FN 50)
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	IV A91 (A091)
Storage Buffer:	Aqueous buffered solution containing ≤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.

The antibody was conjugated to BD Horizon BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome of BD Horizon BUV395 with an Ex Max of 348-nm and an acceptor dye with an Em Max at 737-nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 filter. Due to the excitation of the acceptor dye by other laser lines, there may be significant spillover into channels detecting Alexa Fluor® 700-like dyes (e.g., 712/20-nm filter).

Due to spectral differences between labeled cells and beads, using BD™ CompBeads can result in incorrect spillover values when used with BD Horizon BUV737 reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended. Different BUV737 reagents (e.g., CD4 vs. CD45) can have slightly different fluorescence spillover therefore, it may also be necessary to use clone specific compensation controls when using these reagents.



Flow cytometric analysis of CD69 expressed on stimulated peripheral blood mononuclear cells. *Human PBMC were stimulated for 24 hours with Phytohemagglutinin. The cells were then stained with either BD Horizon™ BUV737 Mouse IgG1, κ Isotype control (Cat. No. 564299; dashed line histogram) or BD Horizon BUV737 Mouse Anti-Human CD69 antibody (Cat. No. 564439; solid line histogram). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable activated lymphocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

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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 BD Horizon™ BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

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. 563794/566349).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564299	BUV737 Mouse IgG1, κ Isotype Control	50 µg	X40
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
8. BD Horizon Brilliant Ultraviolet 737 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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