

## Technical Data Sheet

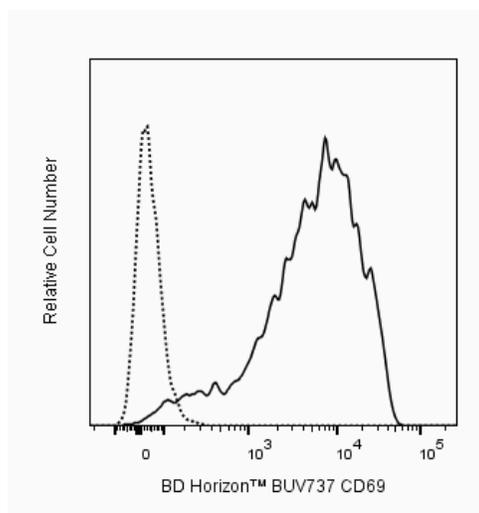
**BUV737 Mouse Anti-Human CD69****Product Information**

<b>Material Number:</b>	<b>612818</b>
<b>Alternate Name:</b>	AIM; CLEC2C; EA1; GP32/28; Leu23; MLR-3; VEA; BL-AC/P26
<b>Size:</b>	25 Tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	FN50 (also known as FN 50)
<b>Immunogen:</b>	Anti-µ stimulated human B lymphocytes
<b>Isotype:</b>	Mouse IgG1, κ
<b>Reactivity:</b>	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
<b>Workshop:</b>	IV A91 (A091)
<b>Storage Buffer:</b>	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 with an Ex Max near 350 nm and an Em Max near 737 nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 nm filter. Due to the excitation of the acceptor dye by the red laser line, there may be significant spillover into red laser detectors with filters in the 700-720 nm range.



**Flow cytometric analysis of CD69 expressed on stimulated peripheral blood mononuclear cells.** Human PBMC were stimulated for 24 hours with Phytohemagglutinin (PHA). The cells were then stained with either BD Horizon™ BUV737 Mouse IgG1, κ Isotype control (Cat. No. 612758; dashed line histogram) or BD Horizon BUV737 Mouse Anti-Human CD69 antibody (Cat. No. 612817/612818; solid line histogram). The fluorescence histogram showing CD69 (or Ig Isotype control staining) were derived from gated events with the forward and side light-scatter characteristics of viable activated lymphocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ Cell Analyzer System and FlowJo™ software.

**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.

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## Application Notes

### Application

Flow cytometry

Routinely Tested

### Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome-conjugated antibodies are bound to BD CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and BD CompBeads. This will ensure that BD CompBeads are appropriate for your specific cellular application.

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) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Note: When using high concentrations of antibody, background binding of this dye to erythroid cell subsets (mature erythrocytes and precursors) has been observed. For researchers studying these cell populations, or in cases where light scatter gating does not adequately exclude these cells from the analysis, this background may be an important factor to consider when selecting reagents for panel(s).

### Suggested Companion Products

Catalog Number	Name	Size	Clone
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
612758	BUV737 Mouse IgG1, $\kappa$ Isotype Control	50 $\mu$ g	X40
612817	BUV737 Mouse Anti-Human CD69	100 Tests	FN50
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  $1 \times 10^6$  cells in a 100- $\mu$ l 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. 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.
6. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
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. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

### References

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