Technical Data Sheet

BUV395 Mouse Anti-Human LAG-3 (CD223)

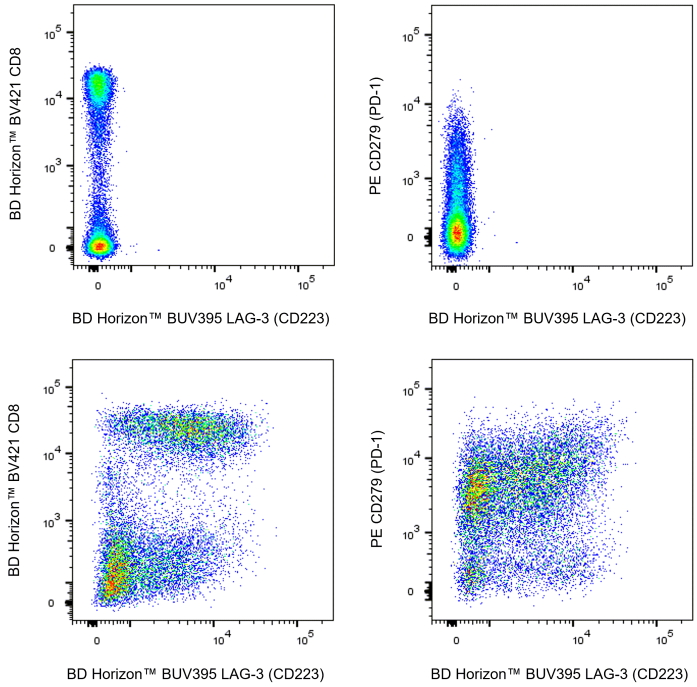
Product Information

Material Number:	**569247**
Alternate Name:	LAG3; CD223; FDC; Lymphocyte activation gene 3 protein; Protein FDC
Entrez Gene ID:	3902
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	T47-530
Immunogen:	Human LAG-3 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The T47-530 specifically recognizes the Lymphocyte Activation Gene 3 (LAG-3) protein which is also known as, Protein FDC, or CD223. LAG-3 is a ~70 kDa type I transmembrane glycoprotein that belongs to the Ig superfamily and exhibits homology to CD4. LAG-3 is expressed on NK cells, regulatory T cells, and activated conventional T cells with higher expression found on CD8+ T cells compared with CD4+ T cells. LAG-3 is an activation induced cell surface molecule that like CD4, binds MHC class II molecules, but with much higher affinity. This may enable LAG-3 to act as a negative competitor of CD4 for MHC class II ligand binding. LAG-3 may associate with the TCR-CD3 complex to downregulate TCR signal transduction and T cell clonal expansion. In contrast, LAG-3-induced signaling may promote dendritic cell activation.

The BD Horizon Brilliant™ Ultraviolet 395 (BUV395) Dye is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This base dye is a polymer fluorochrome with an excitation maximum (Ex Max) of 348-nm and an emission maximum (Em Max) at 395-nm. Driven by BD innovation, BUV395 is designed to be excited by the ultraviolet laser (355-nm) and detected using an optical filter centered near 380-nm (e.g., 379/28-nm bandpass filter). BUV395 is the ideal dye when using only one detector on the ultraviolet laser as it spills into no other detectors and no other fluors spill into it. Please ensure that your instrument's configurations (lasers and optical filters) are appropriate for this dye.



Multicolor flow cytometric analysis of LAG-3 (CD223) expression on unstimulated (Top Plots) and stimulated (Bottom Plots) Human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were cultured for 3 days with plate-bound Anti-Human CD3 (Cat. No. 567109/567108/555329; 10 µg/mL for coating) and soluble Anti-Human CD28 (Cat. No. 555725; 1 µg/mL) antibodies, and Recombinant Human IL-2 Protein (Cat. No. 554603; 10 ng/mL). Unstimulated PBMC (from the same donor) and stimulated PBMC were stained with BD Horizon™ BV421 Mouse Anti-Human CD8 (Cat. No. 562428/562429; Left Plots), PE Mouse Anti-Human CD279 (PD-1) [Cat. No. 560795/561272; Right Plots], and BD Horizon™ BUV395 Mouse Anti-Human LAG-3 (CD223) [Cat. No. 569247] antibodies at 2.0 µg/test. BD Via-Probe™ Cell Viability 7-AAD Solution (Cat. No. 555815/555816) was added to cells right before analysis. Bivariate pseudocolor density plots showing the correlated expression of LAG-3 (CD223) versus CD8 (Left Plots), or LAG-3 (CD223) versus CD279 (PD-1) [Right Plots] were derived from gated events with the forward and side light-scatter characteristics of viable (7-AAD-negative) unstimulated or stimulated lymphocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ X-20 Cell Analyzer System and FlowJo™ software. Data shown on this Technical Data Sheet are not lot specific.*

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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 to the dye under optimum conditions and unconjugated antibody and free dye were removed.

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 to 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 BD Horizon Brilliant™ dyes are used in a multicolor flow cytometry panel. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. When BD Horizon Brilliant Stain Buffer is used in in the multicolor panel, it should also be used in the corresponding compensation controls for all dyes to achieve the most accurate compensation. For the most accurate compensation, compensation controls created with either cells or beads should be exposed to BD Horizon Brilliant Stain Buffer for the same length of time as the corresponding multicolor panel. 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).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
567109	Purified NA/LE Mouse Anti-Human CD3	5 mg	UCHT1
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
554603	Recombinant Human IL-2	10 µg	(none)
563547	BUV395 Mouse IgG1, k Isotype Control	50 µg	X40
562428	BV421 Mouse Anti-Human CD8	100 Tests	RPA-T8
560795	PE Mouse anti-Human CD279 (PD-1)	100 Tests	EH12.1
555815	Cell Viability Solution	500 Tests	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. BD Horizon Brilliant Ultraviolet 395 is covered by one or more of the following US patents: 8,158,444; 8,575,303; 8,354,239.
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. Human donor specific background has been observed in relation to the presence of anti-polyethylene glycol (PEG) antibodies, developed as a result of certain vaccines containing PEG, including some COVID-19 vaccines. We recommend use of BD Horizon Brilliant™ Stain Buffer in your experiments to help mitigate potential background. For more information visit https://www.bdbiosciences.com/en-us/support/product-notices.
9. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).

References

Casati C, Camisaschi C, Novellino L, et al. Human lymphocyte activation gene-3 molecules expressed by activated T cells deliver costimulation signal for dendritic cell activation. *J Immunol.* 2008; 180(6):3782-3788. (Biology)

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Triebel F, Hacene K, Pichon MF. A soluble lymphocyte activation gene-3 (sLAG-3) protein as a prognostic factor in human breast cancer expressing estrogen or progesterone receptors. *Cancer Lett.* 2006; 235(1):147-153. (Biology)

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