Human LAG-3 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF2319

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human LAG-3 in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant mouse LAG-3 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human LAG-3 Leu23-Leu450 Accession # P18627
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

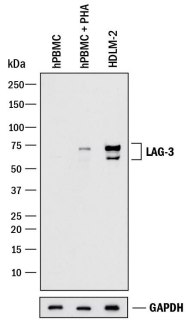
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
CyTOF-reported	Lowther, D.E. *et al.* (2016) JCI Insight **1**:e85935. Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

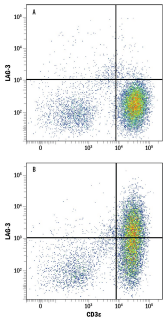
DATA

Western Blot



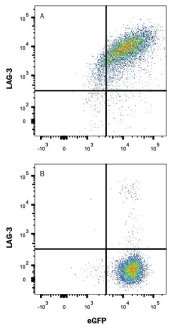
Detection of Human LAG-3 by Western Blot. Western blot shows lysates of human peripheral blood mononuclear cells (PBMC) untreated or treated (+) with 1 ug/mL PHA for 5 days and HDLM-2 human Hodgkin's lymphoma cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human LAG-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2319) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for LAG-3 at approximately 60-75 kDa (as indicated). GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of LAG-3 in $CD3^+$ Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) either (A) untreated or (B) treated with 1 ug/mL PHA for 5 days were stained with Goat Anti-Human LAG-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2319) followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108) and Mouse Anti-Human CD3ε PE-conjugated Monoclonal Antibody (Catalog # FAB100P). Quadrant markers were set based on control antibody staining (Catalog # AB-108-C). View our protocol for Staining Membrane-associated Proteins.

Flow Cytometry



Detection of LAG-3 in HEK293 Human Cell Line Transfected with Human LAG-3 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with either (A) human LAG-3 or (B) irrelevant transfectants and eGFP was stained with Goat Anti-Human LAG-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2319) followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). Quadrant markers were set based on control antibody staining(Catalog # AB-108-C, data not shown). View our protocol for Staining Membrane-associated Proteins.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

LAG-3 (Lymphocyte activation gene-3), also known as CD223, is a member of the immunoglobulin superfamily (IgSF). The mature LAG-3 protein is a 496 amino acid (aa) membrane protein with a 421 aa extracellular region which contains four IgSF domains, a 21 aa transmembrane region and a 54 aa cytoplasmic region. LAG-3 and CD4 molecules share < 20% aa sequence homology but have a similar structure (1, 2). Both molecules bind to MHC class II. LAG-3 binds to MHC class II with higher affinity compared to CD4. Both LAG-3 and CD4 genes are located on the distal part of the short arm of chromosome 12. LAG-3 is an activation-induced molecule, expressed on activated T cells and NK cells, but not on resting T cells. Studies using LAG-3 $^{-/-}$ mice have shown significant delay of T cell apoptosis following antigen stimulation and increased size of memory T cells pool following infection (3, 4). It also has been reported that anti-LAG-3 antibodies up-regulate T cell activation by blocking interaction of LAG-3 and MHC class II. The study has demonstrated that LAG-3 is selectively expressed on activated $CD4^{+}CD25^{+}$ T_{Reg} cells and plays a role in their suppressive activity (5). This evidence indicated, unlike the interaction of CD4 with MHC class II that plays a positive role in T cell activation, LAG-3 binds to MHC class II and negatively regulates T cell activation through LAG-3 signaling. On the other hand, studies have shown that binding of LAG-3 to MHC class II molecules on antigen presenting cells induce maturation of dendritic cells and cytokine secretion by monocytes through MHC class II signal transduction (6). Taken together, LAG-3 may have two major functions, it negatively regulates T cells activation through LAG-3 signaling and stimulates antigen presenting cells which express MHC class II.

References:

1. Triebel, F. *et al.* (1990) J. Exp. Med. **171**:1393.
2. Baixeras, E. *et al.* (1992) J. Exp. Med **176**:327.
3. Workman, C.J. and D.A. Vignali (2003) Eur. J. Immunol. **33**:970.
4. Workman, C.J. *et al.* (2004) J. Immunol. **172**:5450.
5. Huang, C.T. *et al.* (2004) Immunity **21**:503.
6. Andreae, S. *et al.* (2003) Blood **102**:2130.

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