Human LAG-3 Antibody

Monoclonal Mouse IgG_{2B} Clone # 874512

Catalog Number: MAB23196

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human LAG-3 in direct ELISAs.
Source	Monoclonal Mouse IgG_{2B} Clone # 874512
Purification	Protein A or G purified from hybridoma culture supernatant
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.

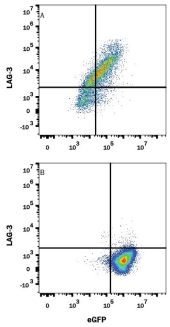
APPLICATIONS

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
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	5-25 µg/mL	Immersion fixed paraffin-embedded sections of human spleen and mouse spleen
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

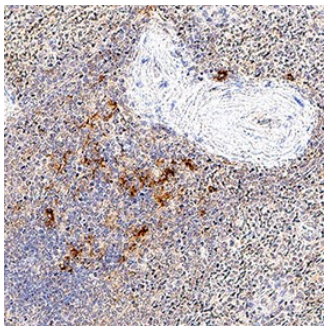
DATA

Flow Cytometry



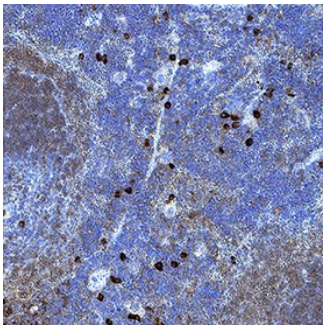
Detection of LAG-3 in HEK293 Human Cell Line Transfected with Human LAG-3 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with either (A) human LAG-3 or (B) irrelevant transfectants and eGFP was stained with Mouse Anti-Human LAG-3 Monoclonal Antibody (Catalog # MAB23196) followed by APC-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # F0101B). Quadrant markers were set based on control antibody staining (Catalog # Catalog # MAB0041, data not shown). View our protocol for Staining Membrane-associated Proteins.

Immunohistochemistry



LAG-3 in Human Spleen. LAG-3 was detected in immersion fixed paraffin-embedded sections of human spleen using Mouse Anti-Human LAG-3 Monoclonal Antibody (Catalog # MAB23196) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry



LAG-3 in Mouse Spleen. LAG-3 was detected in immersion fixed paraffin-embedded sections of mouse spleen using Mouse Anti-Human LAG-3 Monoclonal Antibody (Catalog # MAB23196) at 15 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. Staining was performed our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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Human LAG-3 Antibody

Monoclonal Mouse IgG_{2B} Clone # 874512

Catalog Number: MAB23196

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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.

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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