

**DESCRIPTION**

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human LAG-3 in direct ELISA.
<b>Source</b>	Monoclonal Mouse IgG <sub>2B</sub> Clone # 874528
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant human LAG-3 Leu23-Leu450 Accession # P18627
<b>Formulation</b>	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.

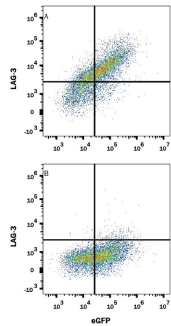
**APPLICATIONS**

**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.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells	See Below
<b>Immunohistochemistry</b>	8-25 µg/mL	Immersion fixed paraffin-embedded sections of mouse spleen
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

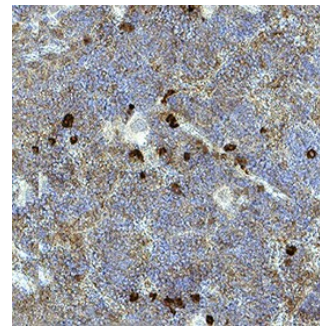
**DATA**

**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 # MAB23197) 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 Mouse Spleen.** LAG-3 was detected in immersion fixed paraffin-embedded sections of mouse spleen using Mouse Anti-Human LAG-3 Monoclonal Antibody (Catalog # MAB23197) 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 using our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.5 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**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<sup>-/-</sup> 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<sup>+</sup>CD25<sup>+</sup> T<sub>Reg</sub> 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. Andrae, S. *et al.* (2003) *Blood* **102**:2130.