abcam

ab237720

Anti-LAG-3/CD223 antibody [CAL77]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.05% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	CAL77
Purification technique	Affinity purification Protein A
Specificity	IHC-P is only recommended for human samples. We obtained negative staining results with mouse.
Concentration	0.52 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purity is greater than 99%.

Reactivity data

IP

Not recommended

Species	Mouse, Human

Dilution info	-
Notes	-

WB

Not recommended

Species	Mouse, Human
Dilution info	-
Notes	-

ICC/IF

Not recommended

Species	Mouse, Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Species	Human
Dilution info	1/500
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/900
Notes	

Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information LAG-3/CD223

Function

Lymphocyte activation gene 3 protein. Inhibitory receptor on antigen activated T-cells (PubMed:20421648, PubMed:35761082, PubMed:7805750, PubMed:8647185). Delivers inhibitory signals upon binding to ligands, such as MHC class II, its main ligand present at the surface of antigen-presenting cells (APCs), and FGL1, which is secreted by hepatocytes and certain types of tumor cells (PubMed:30580966, PubMed:32920841, PubMed:35761082, PubMed:39671469, PubMed:7589152, PubMed:8647185, PubMed:9159144). Ligand-binding initiates a signaling that inhibits the T-cell receptor (TCR) in the immunological synapse, preventing T-cell activation (PubMed:40101708). Mechanistically, ligand-binding promotes (1) ubiquitination of the KIEELE motif, unleashing the RRFSALE motif from the membrane and (2) leading to the formation of condensates with the TCR component CD3E, thereby disrupting the association between CD3E and LCK and preventing TCR activation (PubMed:40101708, PubMed:40592325). May inhibit antigen-specific T-cell activation in synergy with PDCD1/PD-1 (By similarity). Negatively regulates the proliferation, activation, effector function and homeostasis of both CD8(+) and CD4(+) T-cells (PubMed:20421648, PubMed:7805750, PubMed:8647185). Also mediates immune tolerance: constitutively expressed on a subset of regulatory T-cells (Tregs) and contributes to their suppressive function (By similarity). Also acts as a negative regulator of plasmacytoid dendritic cell (pDCs) activation (By similarity). The LAG3-mediated inhibitory pathway is exploited by tumors to attenuate anti-tumor immunity and escape destruction by the immune system, thereby facilitating tumor survival (PubMed:35981087, PubMed:40101708). The blockage of the LAG3- and PDCD1-mediated pathways results in the reversal of the exhausted T-cell phenotype and the normalization of the anti-tumor response, providing a rationale for cancer immunotherapy (PubMed:35981087, PubMed:40101708). Secreted lymphocyte activation gene 3 protein. May function as a ligand for MHC class II (MHC-II) on antigen-presenting cells (APC), promoting APC activation/maturation and driving Th1 immune response.

Storage

Shipped at conditions	Conditional Ambient

Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	LAG-3 also known as CD223 is a transmembrane protein with a mass of approximately 70 kDa. It acts as a checkpoint molecule similar to CTLA-4 and PD-1 in the immune system. LAG-3 is expressed on activated T cells natural killer cells and some dendritic cells. It interacts with its ligand MHC class II leading to inhibition of cellular proliferation and cytokine secretion an important part of immune response regulation.
Biological function summary	LAG-3 is involved in maintaining immune homeostasis and preventing autoimmunity. It is not part of a complex but works in synergy with other immune checkpoint molecules. When interacting with MHC class II LAG-3 transmits inhibitory signals that attenuate immune cell responses. Its role in immune regulation helps to balance immune activation and tolerance which is essential for preventing tissue damage while protecting against pathogens.
Pathways	The inhibitory role of LAG-3 integrates into the T cell receptor (TCR) signaling pathway and is also linked to the PD-1 signaling pathway. In TCR signaling LAG-3 negatively regulates T cell activation. LAG-3 is functionally related to proteins like PD-1 and CTLA-4 sharing similar roles in immune checkpoint pathways that are critical for maintaining immune balance.
Associated diseases and disorders	LAG-3 has links to cancer and autoimmune diseases. In cancer LAG-3 expression on tumor-infiltrating lymphocytes correlates with immune evasion by tumors. This relationship appears in various cancers including melanoma and renal cell carcinoma. In autoimmune diseases LAG-3's expression on regulatory T cells helps control conditions such as multiple sclerosis. It collaborates with CTLA-4 in modulating immune responses that prevent excessive immune activation and tissue damage associated with autoimmunity.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

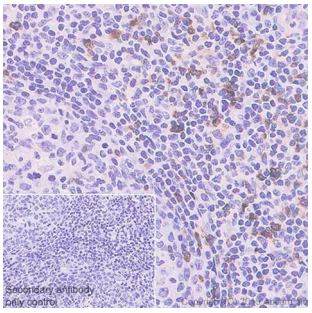
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

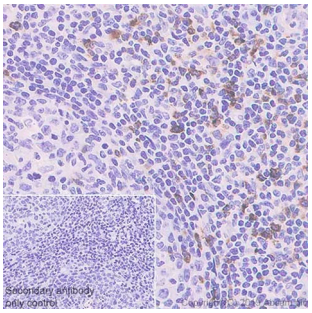
6 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3/CD223 antibody [CAL77] (ab237720)

LAG-3/CD223 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human tonsil using rabbit Anti-LAG-3/CD223 antibody

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling LAG-3 with ab237720 at 1/900 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on the human tonsil is observed. The section was incubated with ab237720 for 15 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP). Perform heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 30 mins.

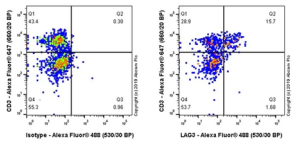


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3/CD223 antibody [CAL77] (ab237720)

LAG-3/CD223 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-LAG-3/CD223 antibody

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling LAG-3 with ab237720 at 1/900 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on the human tonsil is observed. The section was incubated with ab237720 for 15 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead

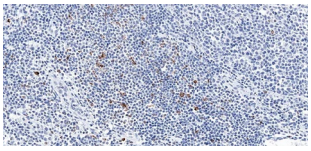
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Flow Cytometry (Intracellular) - Anti-LAG-3/CD223 antibody [CAL77] (ab237720)

LAG-3/CD223 Flow Cytometry (Intracellular) staining using rabbit Anti-LAG-3/CD223 antibody

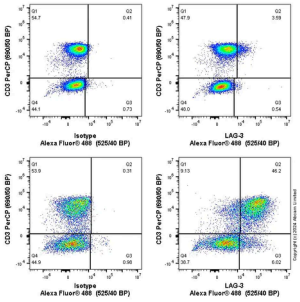
Flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized human peripheral blood mononuclear cell (PBMC) treated with 10µg/ml PHA for 2 days, labeling LAG-3 with ab237720 at 1/500 (right panel) compared with a Rabbit monoclonal IgG (ab172730) (left panel). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody. Cells were surface stained with anti-CD3 conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min followed by intracellular staining with rabbit IgG (Left) and ab237720 (Right).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3/CD223 antibody [CAL77] (ab237720)

LAG-3/CD223 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-LAG-3/CD223 antibody

Formalin-fixed, paraffin-embedded human tonsil tissue stained for LAG-3 with ab237720 at 0.25 µg/ml in immunohistochemical analysis.



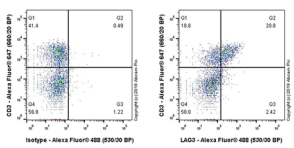
Flow Cytometry (Intracellular) - Anti-LAG-3/CD223 antibody [CAL77] (ab237720)

LAG-3/CD223 Flow Cytometry (Intracellular) staining of Human peripheral blood mononuclear cells using rabbit Anti-LAG-3/CD223 antibody

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) (top) or PBMCs treated with 5 µg/ml phytohaemagglutinin (PHA) for 48 hours (bottom), with ab237720 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). Cells were fixed and permeabilised with BD Cytofix/Cytoperm™ for 20 min. PBMCs were incubated for 30 min on ice in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab237720 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10^6in 100 µl at 0.2 µg/ml (1/12750)) for 30 min at 22°C . The cells were simultaneously stained with CD3.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on live cells.



Flow Cytometry (Intracellular) - Anti-LAG-3/CD223 antibody [CAL77] (ab237720)

LAG-3/CD223 Flow Cytometry (Intracellular) staining using rabbit Anti-LAG-3/CD223 antibody

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1%Tween-20 permeabilized mouse splenocytes treated with 2.5µg/ml Concanavalin A (ConA) for 3 days, labeling LAG-3 with ab237720 at 1/500 (right panel) compared with a Rabbit monoclonal IgG (ab172730) (left panel). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody. Cells were surface stained with anti-CD3 conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min followed by intracellular staining with rabbit IgG (Left) and ab237720 (Right).

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