abcam

ab237718

Anti-LAG-3/CD223 antibody [CAL25]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.5 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	CAL25
Purification technique	Affinity purification Protein A
Concentration	0.513 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purity >99%.

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Not recommended

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	0.3 µg/mL
Notes	-

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

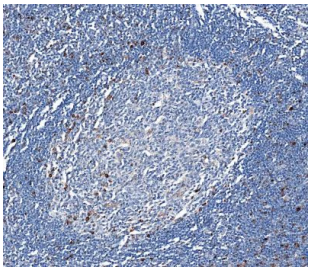
We do not recommend this combination. It is not covered by our product promise.

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.

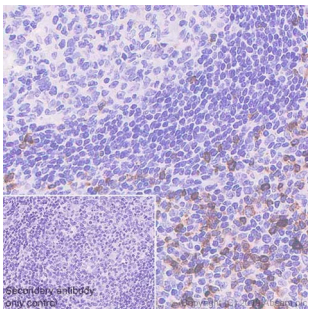
7 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3/CD223 antibody [CAL25] (ab237718)

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

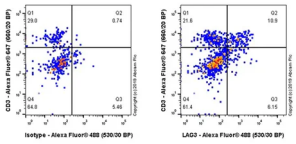
Formalin-fixed, paraffin-embedded human tonsil tissue stained for LAG-3 using ab237718 at 0.3 µg/ml in immunohistochemical analysis.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3/CD223 antibody [CAL25] (ab237718)

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

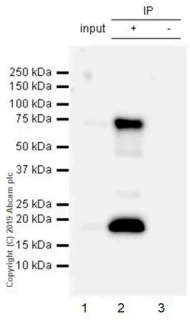
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling LAG-3 using ab237718 at 1/800 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on the human tonsil is observed. The section was incubated with ab237718 for 15 mins at room temperature. 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).



Flow Cytometry (Intracellular) - Anti-LAG-3/CD223 antibody [CAL25] (ab237718)

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 cells (PBMC) treated with 10µg/ml PHA for 48h labeling LAG-3 with ab237718 at 1/50 dilution (Right) compared to a Rabbit monoclonal IgG (ab172730, Left). Goat anti rabbit IgG (Alexa Fluor® 488, ab150097) was used as the secondary at 1/5000 dilution.
Cells were surface stained with anti-CD3 conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min followed by intracellular staining rabbit IgG (Left) or ab237718 (Right).



Immunoprecipitation - Anti-LAG-3/CD223 antibody [CAL25] (ab237718)

LAG-3 was immunoprecipitated from 0.35 mg HEK-293T (human embryonic kidney epithelial cell) transfected with GFP-tagged LAG-3 expression vector whole cell lysate using ab237718 at 1/30 dilution. Western blot was performed on the immunoprecipitate using ab237718 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used as the secondary antibody at 1/5000 dilution.
Lane 1: HEK-293T transfected with GFP-tagged LAG-3 expression vector whole cell lysate 10 µg (input).
Lane 2: ab237718 IP in HEK-293T GFP-tagged LAG-3 expression vector whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab237718 in 293T transfected with GFP-tagged LAG-3 expression vector whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

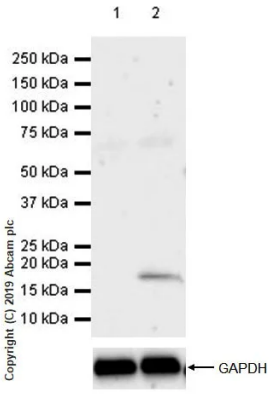
Exposure time: 30 seconds.

This antibody also detects a 16kDa cleaved fragment. (PMID: 15557174)

All lanes:
Immunoprecipitation - Anti-LAG-3/CD223 antibody [CAL25] (ab237718)

Predicted band size: 57 kDa

Observed band size: 16 kDa, 70 kDa



Western blot - Anti-LAG-3/CD223 antibody [CAL25] (ab237718)

LAG-3/CD223 Western blot staining using rabbit Anti-LAG-3/CD223 antibody

Blocking buffer and concentration: 5% NFDM/TBST
This antibody also detects a 16kDa cleaved fragment. (PMID: 15557174)

All lanes:
Western blot - Anti-LAG-3/CD223 antibody [CAL25] (ab237718) at 1/1000 dilution

Lane 1:
Human peripheral blood mononuclear cell (PBMC) whole cell lysate at 20 µg

Lane 2:
Human peripheral blood mononuclear cell (PBMC) treated with 10µg/ml PHA for 48h whole cell lysate at 20 µg

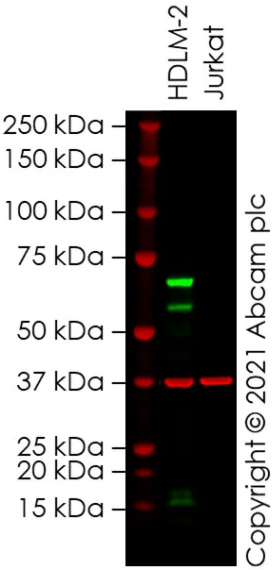
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 57 kDa

Observed band size: 16 kDa, 70 kDa

Exposure time: 3min



Western blot - Anti-LAG-3/CD223 antibody [CAL25] (ab237718)

LAG-3/CD223 Western blot staining using rabbit Anti-LAG-3/CD223 antibody

False colour image of Western blot: Anti-LAG-3 antibody [CAL25] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab237718 was shown to bind specifically to LAG-3. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-LAG-3/CD223 antibody [CAL25] (ab237718) at 1/1000 dilution

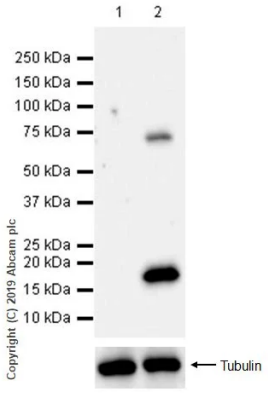
Lane 1:
HDLM-2 cell lysate at 20 µg

Lane 2:
Jurkat cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 57 kDa

Observed band size: 57 kDa, 70 kDa



Western blot - Anti-LAG-3/CD223 antibody [CAL25] (ab237718)

LAG-3/CD223 Western blot staining using rabbit Anti-LAG-3/CD223 antibody

Blocking/diluting buffer and concentration: 5% NFDM/TBST
This antibody also detects a 16kDa cleaved fragment. (PMID: 15557174)

All lanes:
Western blot - Anti-LAG-3/CD223 antibody [CAL25] (ab237718) at 1/1000 dilution

Lane 1:
HEK-293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 2:
HEK-293T transfected with GFP-tagged LAG-3 expression vector whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 57 kDa

Observed band size: 16 kDa, 70 kDa

Exposure time: 3min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.