abcam

ab209236

Anti-LAG-3/CD223 antibody [EPR20261]

Anti-LAG-3/CD223 antibody [EPR20261] (ab209236) is a rabbit monoclonal antibody detecting LAG-3/CD223 in **Western Blot, Flow Cytometry, IP, IHC-P, ICC/IF**. Suitable for **Human**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 10 publications

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR20261
Purification technique	Affinity purification Protein A
Concentration	0.5 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human

Dilution info	1/30
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/500
Notes	-

WB

Tested

Species	Human
Dilution info	1/500
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/1000
Notes	Different mRNA expression levels of LAG3 in brain have been reported in the literature (PMID: 1692078; PMID: 12825348). In IHC, under our experimental conditions, this antibody showed no positive staining on human cerebral cortex.

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

Notes

What is this antibody validated in?
Anti-LAG-3 antibody [EPR20261] (ab209236) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of LAG-3?
Anti-LAG-3 [EPR20261] (ab209236) specifically detects a band for LAG-3 (UniProt: P18627) at a molecular weight of 54kDa.

Trusted by the scientific community
Anti-LAG-3 [EPR20261] (ab209236) was first used in a scientific publication in 2017 and has been cited over 10 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [EPR20261] also available for your convenience: ab209236, PE - ab225484, Alexa Fluor® 488 - ab225485, Alexa Fluor® 647 - ab225486, Carrier free - ab227579, Alexa Fluor® 555 - ab280858, Carrier free - ab282638, Alexa Fluor® 594 - ab283627, ab307517, Carrier free - ab307518

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

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

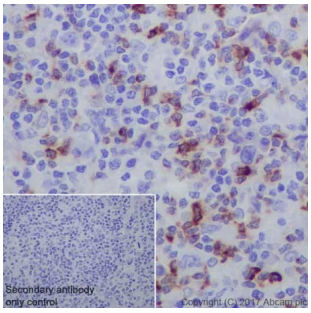
We do not recommend this combination. It is not covered by our product promise.

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.

11 product images



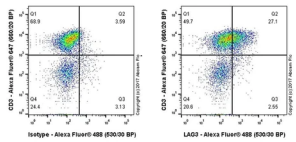
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

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 Hodgkin's lymphoma labeling LAG-3 with ab209236 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on immunocytes of the human Hodgkin's lymphoma [PMID: 11527700; PMID: 16757686].
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

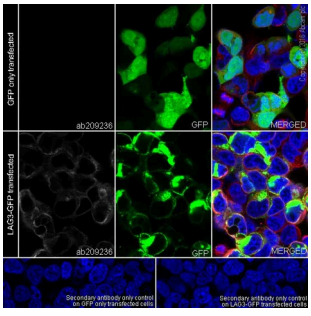
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

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

Flow cytometric analysis of Human peripheral blood mononuclear cells treated with 1 µg/mL PHA for 3 days cells with ab209236 at 1/50 dilution (right) compared with a rabbit monoclonal IgG isotype control (ab172730; left). ab150077 at 1/2000 dilution was used as the secondary antibody. Only the CD3+ population are also positive for LAG3. Gated on total viable cells.



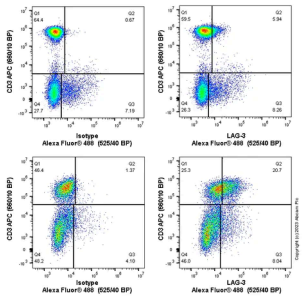
Immunocytochemistry/ Immunofluorescence - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

LAG-3/CD223 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-LAG-3/CD223 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (Human epithelial cell line from embryonic kidney) cells transfected with GFP-tagged LAG3 expression construct or GFP only, labeling LAG-3 with ab209236 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 647) (ab150079) secondary antibody at 1/1000 dilution (green). Confocal image showing positive staining on HEK-293T cells transfected with a GFP-tagged LAG3 expression construct.

The nuclear counter stain is DAPI (blue). Tubulin is detected with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 647) (ab150079) at 1/1000 dilution.



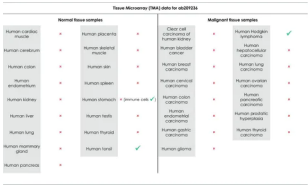
Flow Cytometry - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

LAG-3/CD223 Flow Cytometry staining 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 72 hours (bottom), with ab209236 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). 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 ab209236 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 106 in 100 µl at 5.0 µg/ml (1/402)) for 30min on ice. 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 on ice

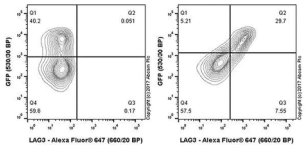
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on viable cells.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

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

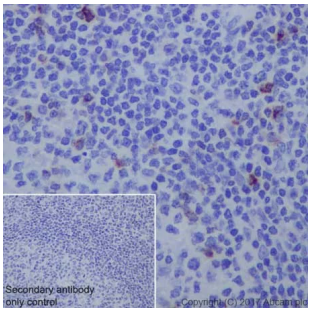
Tissue Microarrays stained for "Anti-LAG-3 antibody [EPR20261]" using "ab209236"in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab209236 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



Flow Cytometry - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

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

Flow cytometric analysis of HEK-293T (Human epithelial cell line from embryonic kidney) cells transfected with a GFP-tagged human LAG-3 construct labeling LAG-3 with ab209236 at 1/500 dilution (right) compared with a rabbit monoclonal IgG isotype control (ab172730; left). Goat anti rabbit IgG (Alexa Fluor® 647) ab150079 at 1/2000 dilution was used as the secondary antibody. Note: Fresh cells without fixation and permeabilization were used to perform FC testing. Only GFP positive population results in LAG3 positive staining (Q2, right panel).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

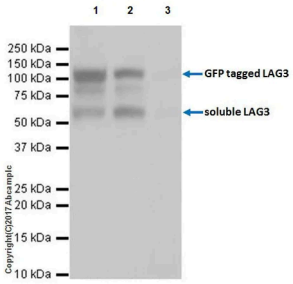
LAG-3/CD223 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-LAG-3/CD223 antibody

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling LAG-3 with ab209236 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Sporadic cytoplasmic staining on immunocytes of human tonsil [PMID: 11527700; PMID: 16757686].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

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



Immunoprecipitation - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

LAG-3 was immunoprecipitated from 0.35 mg of HEK-293T (Human epithelial cell line from embryonic kidney) transfected with a GFP-tagged human LAG3 construct whole cell lysate with ab209236 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab209236 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: HEK-293T transfected with a GFP-tagged human LAG3 construct whole cell lysate 10 µg (Input).

Lane 2: ab209236 IP in HEK-293T transfected with a GFP-tagged human LAG3 construct whole cell lysate.

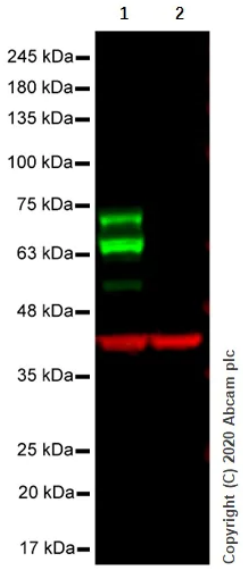
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab209236 in HEK-293T transfected with a GFP-tagged human LAG3 construct whole cell lysate.

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

Exposure time: 1 second.

All lanes:
Immunoprecipitation - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

Predicted band size: 57 kDa



Western blot - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

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

Primary loading control and concentration: Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 1/20000 dilution
Secondary loading control and concentration: Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/10000 dilution

Lanes 1-2: Merged signal (red and green). Green – ab209236 observed at 54-70 kDa. Red - loading control ab8245 observed at 36 kDa.
ab209236 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800RCW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

The expression profile observed in Jurkat is consistent with the literature (PMID: 25108024).

Negative control: Jurkat (PMID: 25108024)

Observed MW: 54-70 kDa

All lanes:

Western blot - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236) at 1/500 dilution

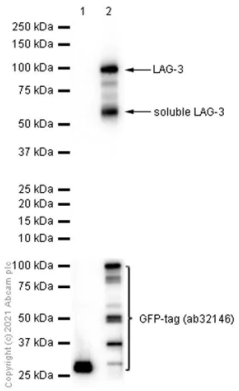
Lane 1:
HDLM-2 (Human Hodgkin lymphoma) whole cell lysate at 20 µg

Lane 2:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 57 kDa



Western blot - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)

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

Blocking/Diluting buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236) at 1/5000 dilution

Lane 1:
293T (Human embryonic kidney epithelial cell) transfected with an empty vector (vector control), containing a GFP tag, whole cell lysate at 15 µg

Lane 2:
293T transfected with LAG3 expression vector containing a GFP tag, whole cell lysate at 15 µg

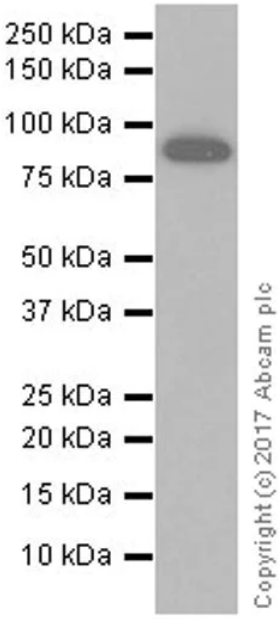
Secondary

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

Predicted band size: 57 kDa

Exposure time: 5s

Western blot - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236)



LAG-3/CD223 Western blot staining of Human LAG3 Fc chimera recombinant protein (aa23-450) using rabbit Anti-LAG-3/CD223 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-LAG-3/CD223 antibody [EPR20261] (ab209236) at 1/1000 dilution

All lanes:
Human LAG3 Fc chimera recombinant protein (aa23-450) at 0.01 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: 90 kDa

Exposure time: 1s

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