abcam

Product datasheet

Anti-LAG-3 antibody [EPR20261] ab209236

Recombinant RabMAb

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Overview

Product name	Anti-LAG-3 antibody [EPR20261]
Description	Rabbit monoclonal [EPR20261] to LAG-3
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HDLM-2 cells and Human LAG3 Fc chimera recombinant protein (aa23-450). 293T transfected with LAG3 expression vector containing a GFP tag, whole cell lysate. IHC-P: Human tonsil and Hodgkin's lymphoma tissues. ICC/IF: HEK-293T cells transfected with a GFP-tagged LAG3 expression construct. Flow Cyt: HEK-293T transfected with a GFP-tagged human LAG3 construct, Human PBMCs. IP: HEK-293T transfected with a GFP-tagged human LAG3 construct whole cell lysate.
General notes	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR20261
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab209236 in the following tested applications.

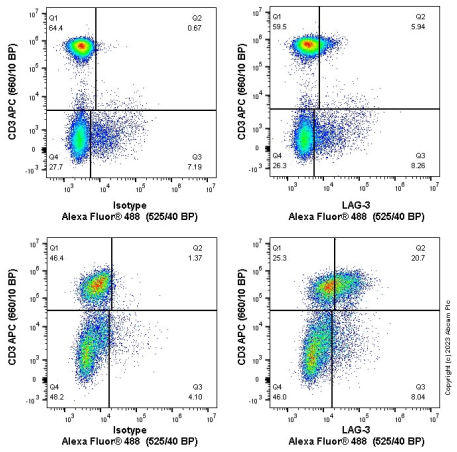
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/500. Detects a band of approximately 70 kDa (predicted molecular weight: 54 kDa).
IHC-P	★★★★☆ (1)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. 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.
ICC/IF		1/100.
Flow Cyt		1/500.
IP		1/30.

Target

Function	Involved in lymphocyte activation. Binds to HLA class-II antigens.
Tissue specificity	On cell surface of activated NK and T-lymphocytes.
Sequence similarities	Contains 3 Ig-like C2-type (immunoglobulin-like) domains. Contains 1 Ig-like V-type (immunoglobulin-like) domain.
Cellular localization	Membrane.

Images

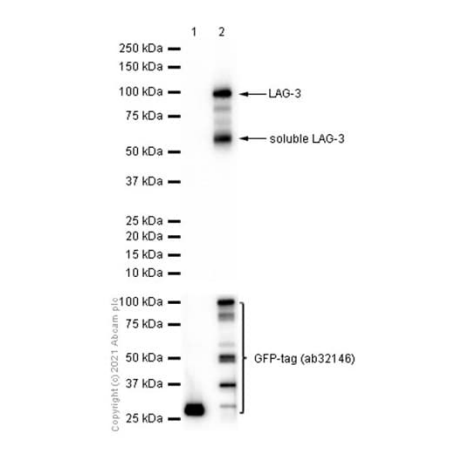


Flow Cytometry - Anti-LAG-3 antibody [EPR20261] (ab209236)

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 10^6 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.



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

All lanes : Anti-LAG-3 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
Lane 2 : 293T transfected with LAG3 expression vector containing a GFP tag, whole cell lysate

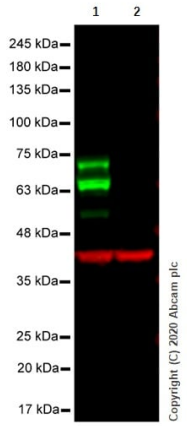
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 54 kDa

Exposure time: 5 seconds

Blocking/Diluting buffer: 5% NFDM/TBST.



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

All lanes : Anti-LAG-3 antibody [EPR20261] (ab209236) at 1/500 dilution

Lane 1 : HDLM-2 (Human Hodgkin lymphoma) whole cell lysate
Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 54 kDa

Primary loading control and concentration: Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) at 1/20000 dilution

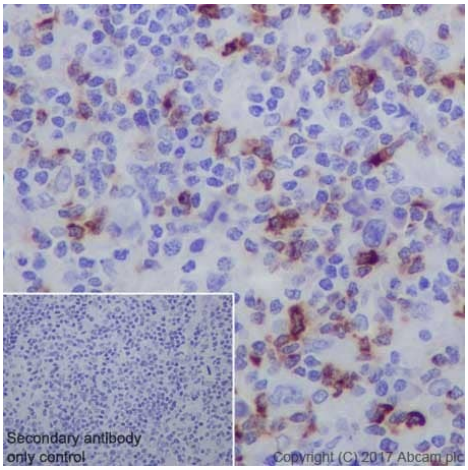
Secondary loading control and concentration: Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) at 1/10000 dilution

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



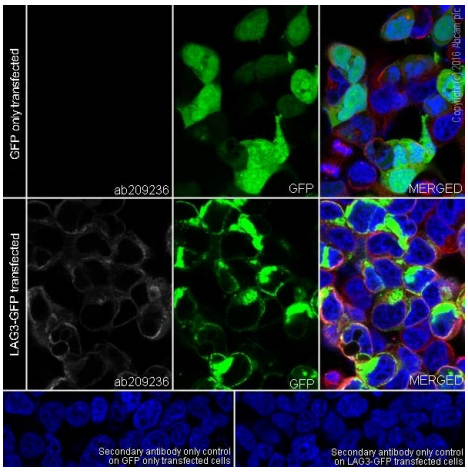
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3 antibody [EPR20261] (ab209236)

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.



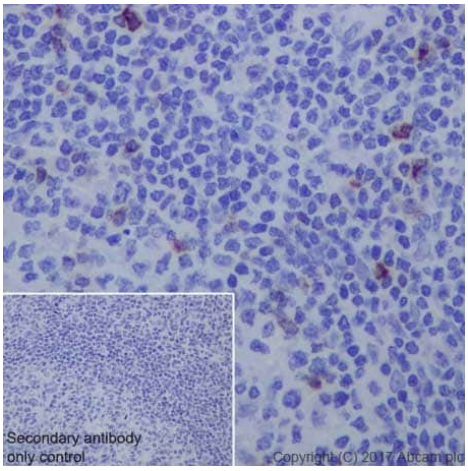
Immunocytochemistry/ Immunofluorescence - Anti-LAG-3 antibody [EPR20261] (ab209236)

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.



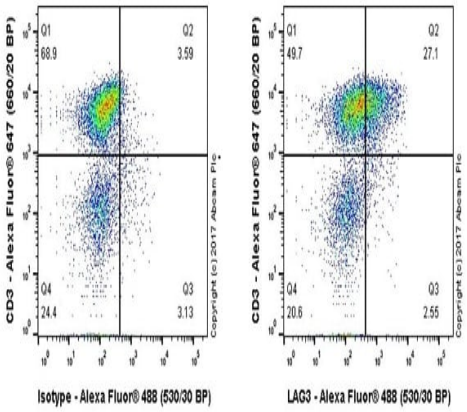
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3 antibody [EPR20261] (ab209236)

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.

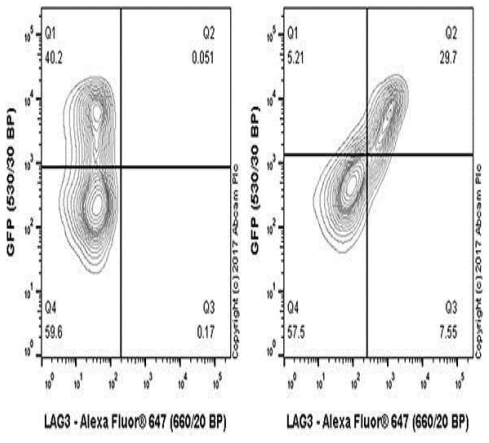
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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

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.

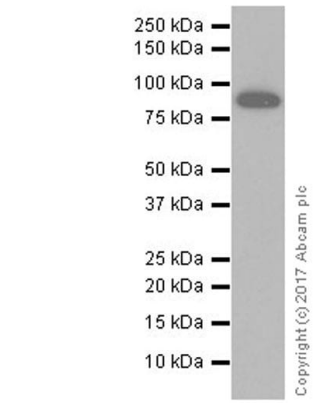
Only the CD3+ population are also positive for LAG3. Gated on total viable cells.



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

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).



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

Anti-LAG-3 antibody [EPR20261] (ab209236) at 1/1000 dilution + Human LAG3 Fc chimera recombinant protein (aa23-450) at 0.01 µg

Secondary

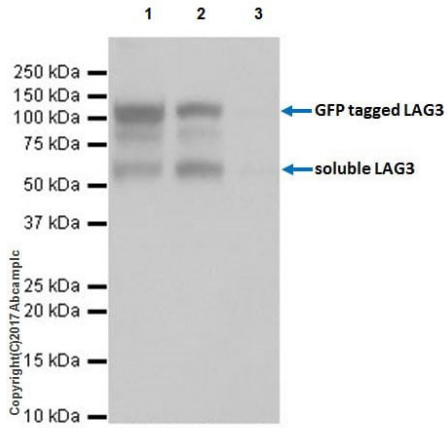
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 54 kDa

Observed band size: 90 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-LAG-3 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.

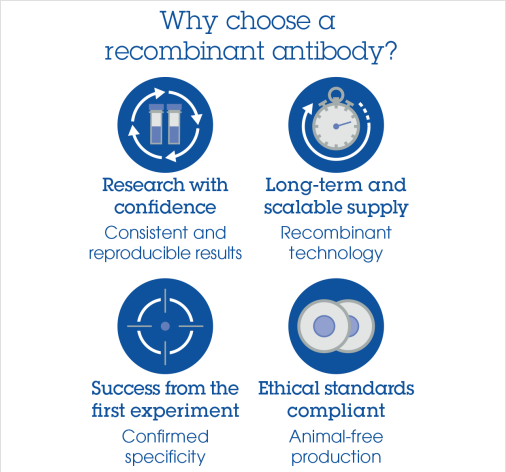
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab209236 in HEK-293T transfected with a GFP-tagged human LAG3 construct whole cell lysate.

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

Exposure time: 1 second.

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

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).



Anti-LAG-3 antibody [EPR20261] (ab209236)

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