

## Product datasheet

# Anti-LAG-3 antibody [EPR20261] ab209236

Recombinant RabMAb

★★★★☆ **1 Abreviews** **10 References** [12 Images](#)

### Overview

<b>Product name</b>	Anti-LAG-3 antibody [EPR20261]
<b>Description</b>	Rabbit monoclonal [EPR20261] to LAG-3
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> WB, IHC-P, ICC/IF, Flow Cyt, IP
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	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.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
<b>Purity</b>	Protein A purified

<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR20261
<b>Isotype</b>	IgG

## Applications

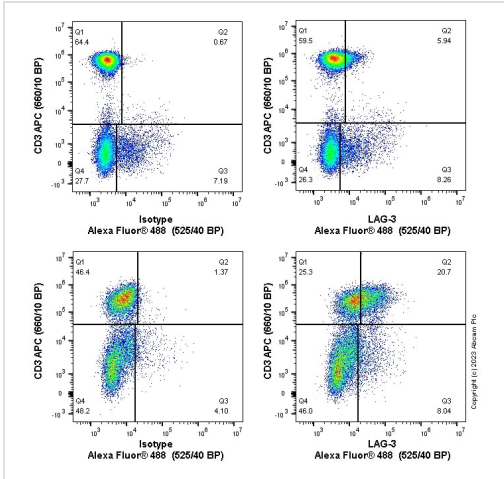
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab209236 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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

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

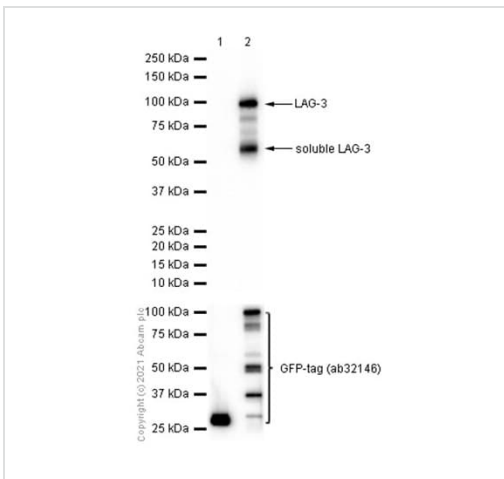
## Images



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<sup>6</sup> 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.

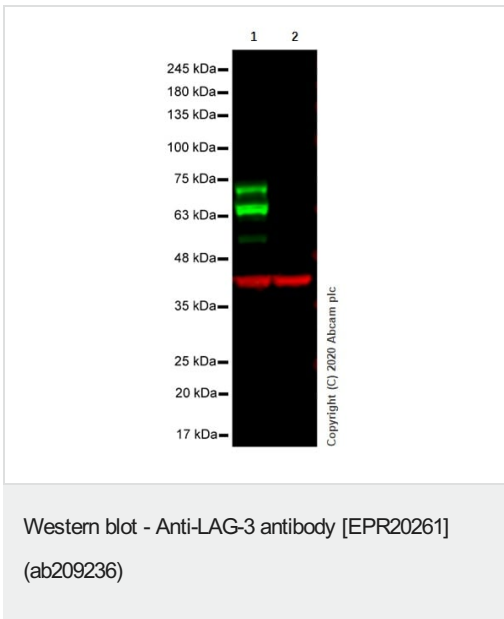
**Secondary**

**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.



**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

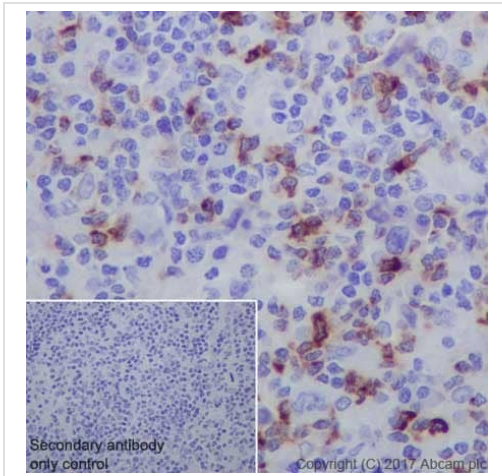
Lanes 1-2: Merged signal (red and green). Green – ab209236 observed at 54-70 kDa. Red - loading control **ab8245** observed at 36 kDa.

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



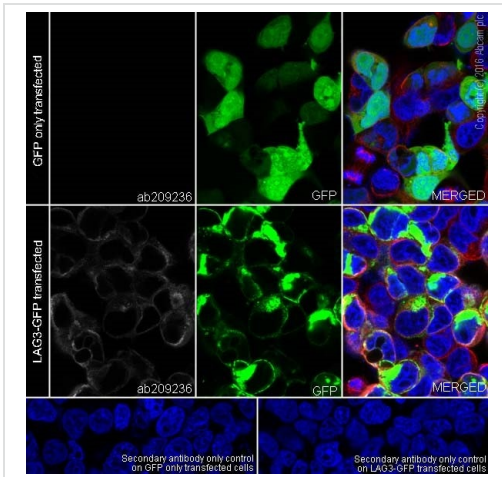
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3 antibody [EPR20261] (ab209236)

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.



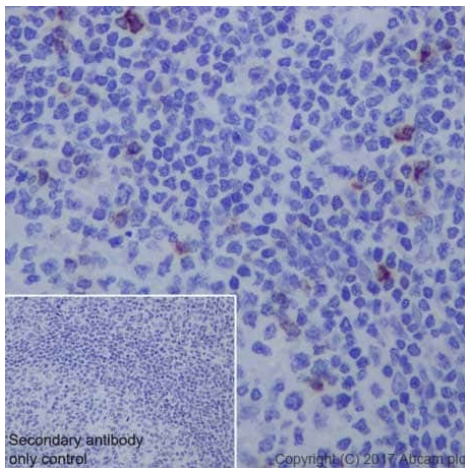
Immunocytochemistry/ Immunofluorescence - Anti-LAG-3 antibody [EPR20261] (ab209236)

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.



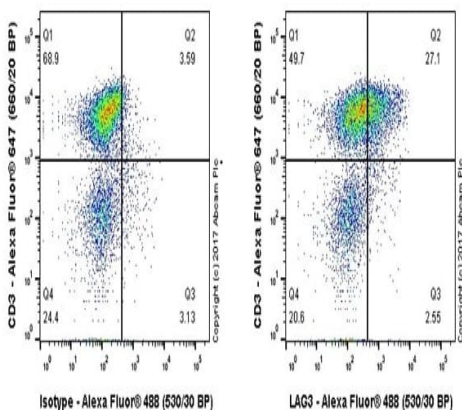
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3 antibody [EPR20261] (ab209236)

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.

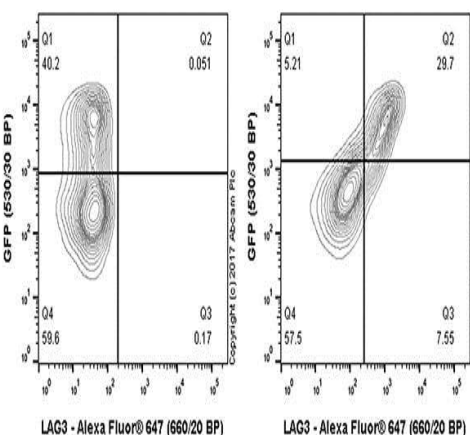
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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.

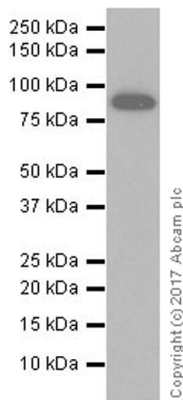


Flow Cytometry - Anti-LAG-3 antibody [EPR20261] (ab209236)

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<sup>®</sup> 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**

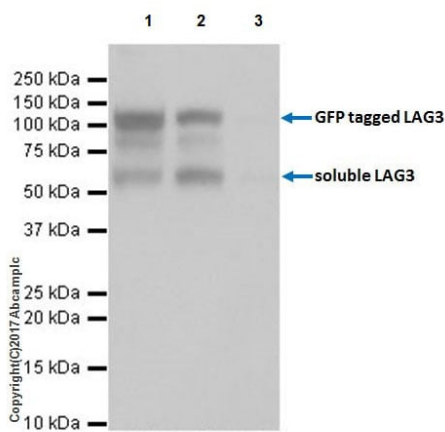
Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

**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.


Tissue Microarray (TMA) data for ab209236

Normal tissue samples			Malignant tissue samples				
Human cardiac muscle	x	Human placenta	x	Clear cell carcinoma of human kidney	x	Human Hodgkin lymphoma	✓
Human cerebrum	x	Human skeletal muscle	x	Human bladder cancer	x	Human hepatocellular carcinoma	x
Human colon	x	Human skin	x	Human breast carcinoma	x	Human lung carcinoma	x
Human endometrium	x	Human spleen	x	Human cervical carcinoma	x	Human ovarian carcinoma	x
Human kidney	x	Human stomach	x (Immune cells ✓)	Human colon carcinoma	x	Human pancreatic carcinoma	x
Human liver	x	Human testis	x	Human endometrial carcinoma	x	Human prostatic hyperplasia	x
Human lung	x	Human thyroid	x	Human gastric carcinoma	x	Human thyroid carcinoma	x
Human mammary gland	x	Human tonsil	✓	Human glioma	x		
Human pancreas	x						

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).

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


**Why choose a recombinant antibody?**




**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

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

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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