abcam

Product datasheet

Anti-LAG-3 antibody [EPR20294-77] ab209238

Recombinant   RabMAb

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Overview

Product name	Anti-LAG-3 antibody [EPR20294-77]
Description	Rabbit monoclonal [EPR20294-77] to LAG-3
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Mouse LAG-3 recombinant protein (aa24-442) tagged with murine IgG2A at the C-terminus; mouse splenocytes treated with 5 ug/ml concanavalin A for 72 hours, whole cell lysate. ICC/IF: Mouse splenocytes. IP: Mouse splenocytes treated with 5 µg/ml concanavalin A for 72 hours, 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, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20294-77

Isotype	IgG

Applications

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

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 54, 70 kDa (predicted molecular weight: 57 kDa).
ICC/IF		1/100.
IP		1/30.

Target

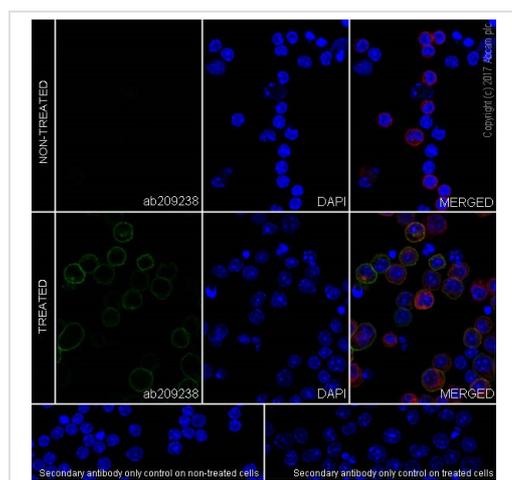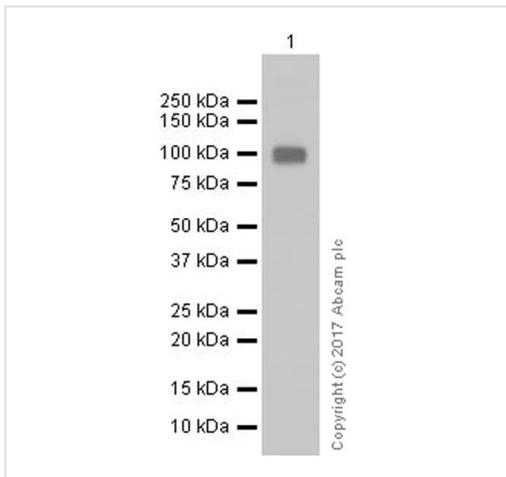|	
Function	Involved in lymphocyte activation. Binds to HLA class-II antigens.
Tissue specificity	On cell surface of activated NK and T-lymphocytes.
Sequence similarities	Contains 3 Ig-like C2-type (immunoglobulin-like) domains. Contains 1 Ig-like V-type (immunoglobulin-like) domain.
Cellular localization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

Images

Immunocytochemistry/ Immunofluorescence - Anti-LAG-3 antibody [EPR20294-77] (ab209238)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized untreated and concanavalin A (2.5 µg/ml for 72 hours) treated mouse splenocytes labeling LAG-3 with ab209238 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing increased membranous staining following treatment of mouse splenocytes with concanavalin A (2.5 µg/ml for 72 hours) (PMID 20391435; PMID 26318293; PMID 11527700; PMID 19631993).

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/1000 dilution

Western blot - Anti-LAG-3 antibody [EPR20294-77] (ab209238)

Anti-LAG-3 antibody [EPR20294-77] (ab209238) at 1/10000 dilution + Mouse LAG-3 recombinant protein (aa24-442) tagged with murine IgG2A at the C-terminus, 10 ng

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution
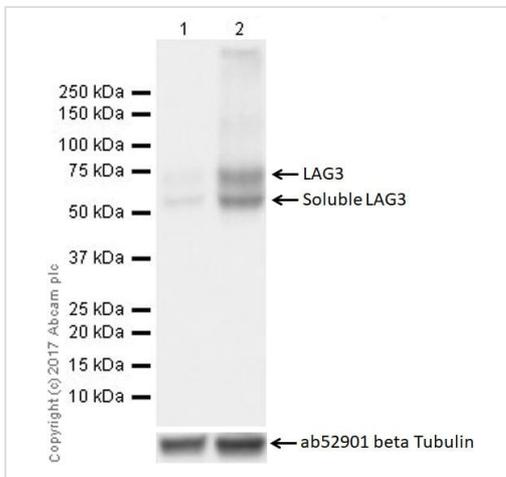
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 57 kDa
Observed band size: 100 kDa

Exposure time: 1 second

Blocking/Dilution Buffer: 5% NFDM/TBST

Western blot - Anti-LAG-3 antibody [EPR20294-77] (ab209238)

All lanes : Anti-LAG-3 antibody [EPR20294-77] (ab209238) at 1/1000 dilution

Lane 1 : Untreated mouse splenocytes whole cell lysate
Lane 2 : Mouse splenocytes treated with 5 ug/ml concanavalin A for 72 hours whole cell lysate

Lysates/proteins at 10 µg per lane.

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

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 57 kDa
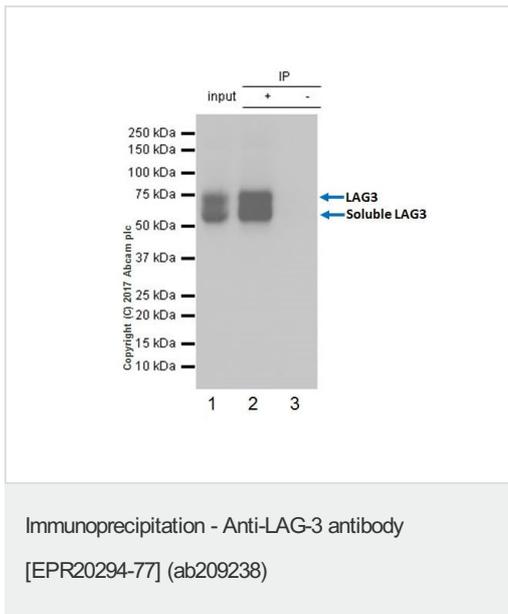**Observed band size:** 54, 70 kDa

Exposure time: 10 seconds

Blocking/Dilution Buffer: 5% NFDM/TBST

The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument.

LAG3 expression can be elevated in activated splenocytes (PMID 19631993).

The molecular mass observed is consistent with the literature (PMID 15971272; PMID 15557174).

Immunoprecipitation - Anti-LAG-3 antibody [EPR20294-77] (ab209238)

LAG-3 was immunoprecipitated from 0.35 mg of mouse splenocytes (treated with 5 µg/ml concanavalin A for 72 hours) whole cell lysate with ab209238 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab209238 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

Lane 1: Mouse splenocytes (treated with 5 µg/ml concanavalin A for 72 hours), whole cell lysate 10µg (Input).

Lane 2: ab209238 IP in mouse splenocytes (treated with 5 µg/ml concanavalin A for 72 hours), whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab209238 in mouse splenocytes (treated with 5 µg/ml concanavalin A for 72 hours), whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 1 second.

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 [EPR20294-77] (ab209238)

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