abcam

Product datasheet

Anti-LAG-3 antibody [CAL25] - BSA and Azide free ab251604

Recombinant RabMAb

6 Images

Overview

Product name	Anti-LAG-3 antibody [CAL25] - BSA and Azide free
Description	Rabbit monoclonal [CAL25] to LAG-3 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, Flow Cyt (Intra), IP, WB **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human tonsil tissue. Flow: Human peripheral blood mononuclear cell (PBMC) treated with 10µg/ml PHA for 48h. IP:HEK-293T transfected with GFP-tagged LAG-3 expression vector whole cell lysate.
General notes	ab251604 is the carrier-free version of **ab237718**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Purification notes	Purity is greater than 99%.
Clonality	Monoclonal
Clone number	CAL25
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab251604 in the following tested applications.

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

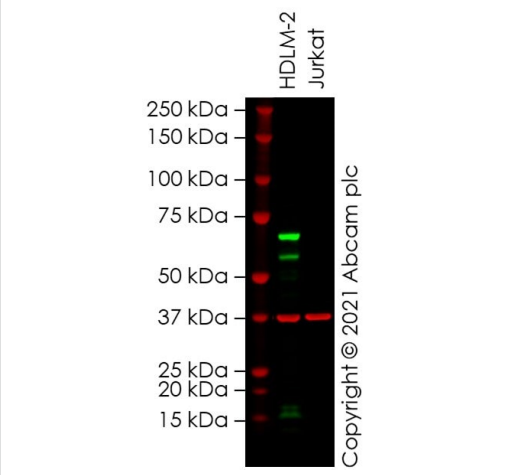
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 57 kDa.

Application notes Is unsuitable for ICC/IF.

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



Western blot - Anti-LAG-3 antibody [CAL25] - BSA and Azide free (ab251604)

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

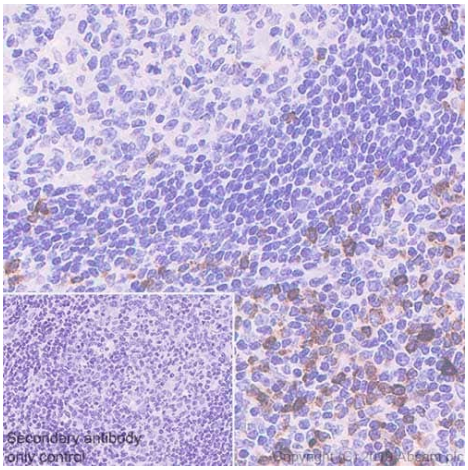
Lane 1 : Wild-type A431 cell lysate
Lane 2 : SOS1 knockout A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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.

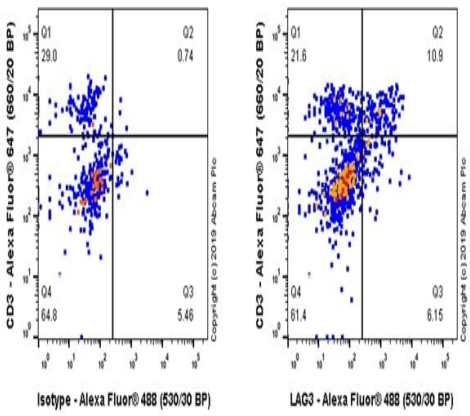


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3 antibody [CAL25] - BSA and Azide free (ab251604)

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).

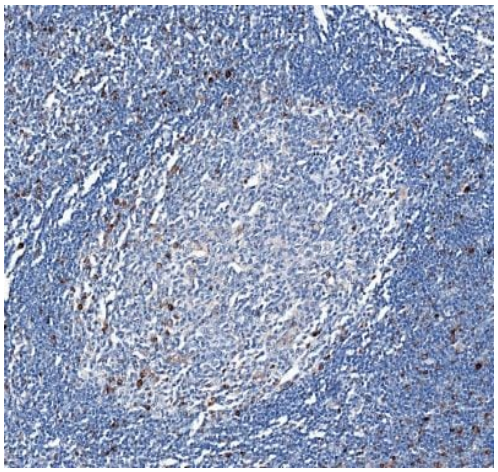
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab237718**).



Flow Cytometry (Intracellular) - Anti-LAG-3 antibody [CAL25] - BSA and Azide free (ab251604)

Intracellular 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).

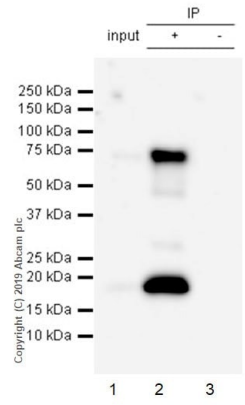
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab237718**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAG-3 antibody [CAL25] - BSA and Azide free (ab251604)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab237718**).



Immunoprecipitation - Anti-LAG-3 antibody [CAL25] - BSA and Azide free (ab251604)

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)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab237718**).

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