abcam

Product datasheet

Anti-Lingo1 antibody ab23631

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Overview

Product name	Anti-Lingo1 antibody
Description	Rabbit polyclonal to Lingo1
Host species	Rabbit
Specificity	Rabbit polyclonal to Lingo1 (ab23631) detects Lingo1 protein at ~83kDa in mouse and human brain lysates. This band is larger than predicted on Swiss Prot (69kDa; Q9D1T0) possibly due to post-translational modification and is consistent with published literature on Lingo1 protein detection in brain lysate. The strong band observed at ~ 17kDa in the mouse brain lysate (lane 1) corresponds to a cleavage fragment of Lingo1 (Swiss Prot IDs: Q3TQJ4)
Tested applications	**Suitable for:** WB, ICC/IF, IHC-Fr, IP, Functional Studies, IHC-FoFr
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Chicken
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 600 to the C-terminus of Human Lingo1. Read Abcam's proprietary immunogen policy (Peptide available as ab25890.)
Positive control	Mouse Brain, Brain (Mouse) Whole Cell Lysate - normal tissue, 0 days old (ab7188) This antibody gave a positive result when used in the following formaldehyde fixed cell lines: SKNSH.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab23631** in the following tested applications.

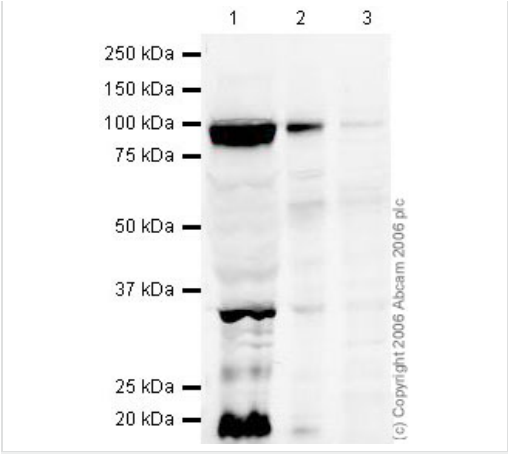
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆	Use a concentration of 1 µg/ml. Detects a band of approximately 83 kDa (predicted molecular weight: 83 kDa).
ICC/IF		Use a concentration of 5 µg/ml.
IHC-Fr		1/500. PubMed: 20407577
IP		Use at an assay dependent concentration.
Functional Studies		Use at an assay dependent concentration. PubMed: 20573699
IHC-FoFr	★★★★☆	1/3000.

Target

Function	Functional component of the Nogo receptor signaling complex (RTN4R/NGFR) in RhoA activation responsible for some inhibition of axonal regeneration by myelin-associated factors. Is also an important negative regulator of oligodentrocyte differentiation and axonal myelination. Acts in conjunction with RTN4 and RTN4R in regulating neuronal precursor cell motility during cortical development.
Tissue specificity	Expressed exclusively in the central nervous system. Highest level in the in amygdala, hippocampus, thalamus and cerebral cortex. In the rest of the brain a basal expression seems to be always present. Up-regulated in substantia nigra neurons from Parkinson disease patients.
Sequence similarities	Contains 1 Ig-like C2-type (immunoglobulin-like) domain. Contains 11 LRR (leucine-rich) repeats. Contains 1 LRRCT domain. Contains 1 LRRNT domain.
Post-translational modifications	N-glycosylated. Contains predominantly high-mannose glycans.
Cellular localization	Cell membrane.

Images



Western blot - Anti-Lingo1 antibody (ab23631)

All lanes : Anti-Lingo1 antibody (ab23631) at 1 µg/ml

Lane 1 : Mouse brain
Lane 2 : Mouse brain tissue lysate - total protein (0 days) (ab7188)
Lane 3 : Human Brain Tissue Lysate

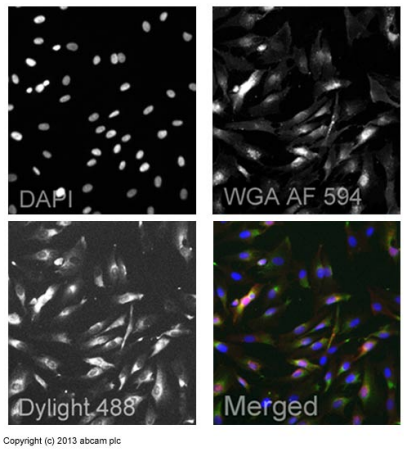
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Rabbit IgG secondary antibody (ab28446) at 1/10000 dilution

Performed under reducing conditions.

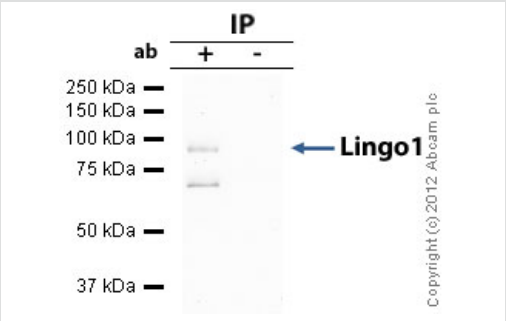
Predicted band size: 83 kDa
Observed band size: 83 kDa
Additional bands at: 17 kDa (possible cleavage fragment), 34 kDa (possible degradation product)

Rabbit polyclonal to Lingo1 (ab23631) detects Lingo1 protein at ~83kDa in mouse and human brain lysates. This band is larger than predicted on Swiss Prot (69kDa; Q9D1T0) possibly due to post-translational modifications and is consistent with published literature on Lingo1 protein detection in brain lysate. The strong band observed at ~ 17kDa in the mouse brain lysate (lane 1) corresponds to a cleavage fragment of Lingo1 (Swiss Prot IDs: Q3TQJ4)



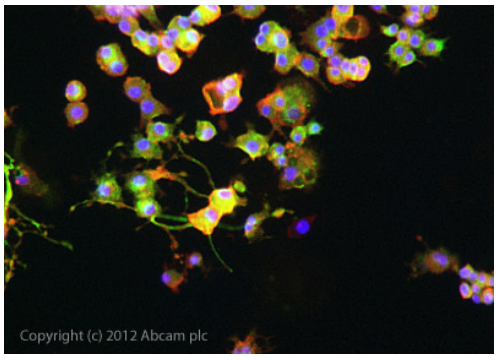
Immunocytochemistry/ Immunofluorescence - Anti-Lingo1 antibody (ab23631)

ICC/IF image of ab23631 stained SKNSH cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab23631 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



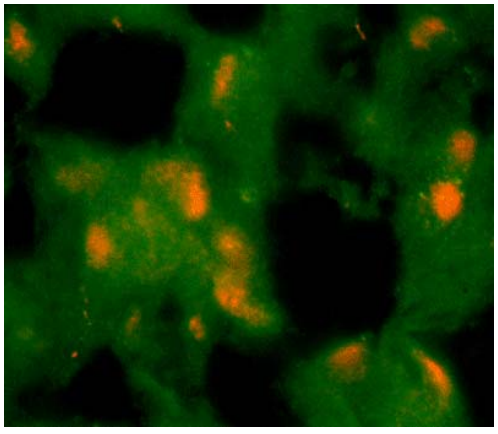
Immunoprecipitation - Anti-Lingo1 antibody (ab23631)

Lingo1 was immunoprecipitated using 0.5mg Mouse Brain whole tissue lysate, 5µg of Rabbit polyclonal to Lingo1 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, Mouse Brain whole tissue lysate lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab23631.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).
Band: 83kDa:Lingo1; non specific - 70kDa: We are unsure as to the identity of this extra band.



Immunocytochemistry/ Immunofluorescence - Anti-Lingo1 antibody (ab23631)

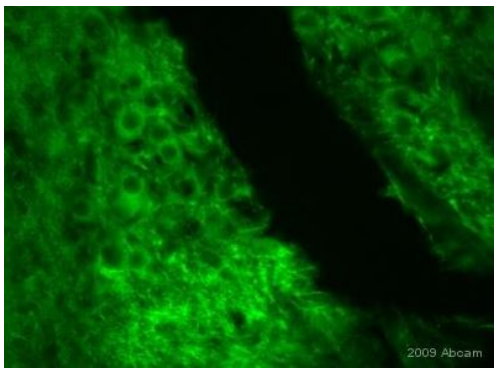
ICC/IF image of ab23631 stained PC12 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab23631 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Frozen sections) - Anti-Lingo1 antibody (ab23631)

Image from Chandrasekar V et al, Front Behav Neurosci. 2010 Apr 5;4:14, Fig 4.

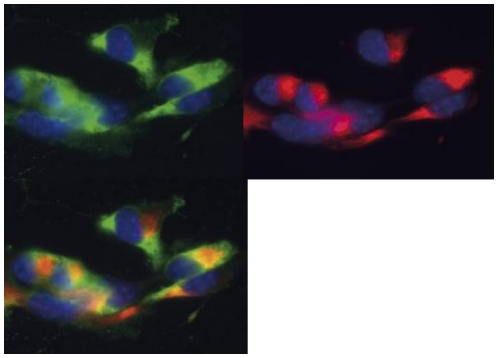
ab23631 staining Lingo1 in in rat brain tissue by Immunohistochemistry (Frozen sections). Rats were decapitated, brains were quickly removed and immediately frozen in isopentane (at -30°C for 3 minutes) and kept at -25°C. Coronal sections were cut at 14 µm in a cryostat and placed on gelatinized glass slides, air-dried at room temperature for 20 minutes and kept at -25°C until further processing. Brain sections were fixed for 10 minutes in cold acetone and washed three times in 1 × phosphate-buffered saline (PBS). Non-specific binding sites were blocked by incubating slices for 1 hour in 1 × PBS containing 1% bovine serum albumin, 1% Triton X-100 and 3% normal goat serum. Sections were then incubated overnight at 4°C with the primary antibody at a 1/500 dilution. The sections were then stained by 30 minutes incubation with Hoechst at a 1/1500 dilution.



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-Lingo1 antibody (ab23631)

This image is courtesy of an Abreview submitted by Sophie Pezet.

Immunohistochemical analysis of rat hypothalamus frozen sections, labeling Lingo1 with ab23631 (diluted 1/3000 in 0.3% Triton X-100 in PBS). Tissues were perfusion fixed with 4% paraformaldehyde and cryoprotected in 30% sucrose. Incubation with ab23631 was for 18 hours at 20°C. ab60314 (undiluted) was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Lingo1 antibody (ab23631)

Images cropped from Lööv et al., PLoS One, 1, e29771, Fig. 1.; DOI: 10.1371/journal.pone.0029771

Immunocytochemical analysis of mouse neural stem and progenitor cells, labeling Lingo1 with ab23631. Cells were fixed in 4% paraformaldehyde, then permeabilized and blocked for 30 minutes in 5% natural goat serum in PBS. Incubation with ab23631 (diluted 1/200) was for 1-4 hours at RT. DAPI was used for staining nuclei.

Top Left: Lingo1 staining

Top Right: Nestin staining

Bottom Left: Merge

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