**Product datasheet**

**Anti-L1CAM antibody [2C2] ab24345**

<table>
<thead>
<tr>
<th>Overview</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product name</strong></td>
<td>Anti-L1CAM antibody [2C2]</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Mouse monoclonal [2C2] to L1CAM</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Mouse</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: ICC/IF, IHC-P, WB</td>
</tr>
<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Rat, Human</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>corresponding to L1CAM.</td>
</tr>
<tr>
<td><strong>Positive control</strong></td>
<td>WB: Mouse whole brain, Rat whole brain and Human whole brain tissue lysates. IHC-P: normal Human kidney and normal Rat kidney tissue sections. ICC/IF: PC12 (undifferentiated and NGF-differentiated) and Neuro-2A (undifferentiated and TRA-differentiated).</td>
</tr>
<tr>
<td><strong>General notes</strong></td>
<td>L1CAM can be detected between 200-220 kD. In brain samples it is typically seen at ~ 200 kD. When the protein is overexpressed in vitro it is often detected as a doublet with bands at 200 and 220 kD. The unglycosylated, unprocessed L1CAM is ~ 140-150 kDa. The protein has 21 putative N-glycosylation sites on the extracellular portion of the protein which, when they are all glycosylated, results in a detected MW of 200-220 kD depending upon how many residues are actually glycosylated. L1CAM can be cleaved by the metalloprotease ADAM10 resulting in fragments of 180 kD and 40 kD. L1CAM can also be cleaved by plasmin resulting in fragments of 140 kD and 80 kD. In theory, therefore, one could detect bands at ~220, 200, 180, 140, 80 and 40 kD.</td>
</tr>
</tbody>
</table>

This product was changed from ascites to tissue culture supernatant on 08/Jul/2019. Lot numbers higher than GR3248431 are from tissue culture supernatant. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As.
Properties

Form
Liquid

Storage instructions

Storage buffer
Preservative: 0.02% Sodium azide
Constituents: PBS, 6.97% L-Arginine

Purity
Protein G purified

Purification notes
Purified from TCS.

Clonality
Monoclonal

Clone number
2C2

Isotype
IgG2b

Applications

The Abpromise guarantee
Our Abpromise guarantee covers the use of ab24345 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐ (3)</td>
<td>Use a concentration of 5 µg/ml.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐ (1)</td>
<td>Use a concentration of 0.1 - 0.5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Unsuitable for IHC-P in mouse tissue from in-house testing.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐ (5)</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 200 kDa. Cleavage products observed at 60-80 kDa.</td>
</tr>
</tbody>
</table>

Target

Function
Cell adhesion molecule with an important role in the development of the nervous system. Involved in neuron-neuron adhesion, neurite fasciculation, outgrowth of neurites, etc. Binds to axonin on neurons.

Involvement in disease
Defects in L1CAM are the cause of hydrocephalus due to stenosis of the aqueduct of Sylvius (HSAS) [MIM:307000]. Hydrocephalus is a condition in which abnormal accumulation of cerebrospinal fluid in the brain causes increased intracranial pressure inside the skull. This is usually due to blockage of cerebrospinal fluid outflow in the brain ventricles or in the subarachnoid space at the base of the brain. In children is typically characterized by enlargement of the head, prominence of the forehead, brain atrophy, mental deterioration, and convulsions. In adults the syndrome includes incontinence, imbalance, and dementia. HSAS is characterized by mental retardation and enlarged brain ventricles.

Defects in L1CAM are the cause of mental retardation-aphasia-shuffling gait-adducted thumbs syndrome (MASA) [MIM:303350]; also known as corpus callosum hypoplasia, psychomotor retardation, adducted thumbs, spastic paraparesis, and hydrocephalus or CRASH syndrome. MASA is an X-linked recessive syndrome with a highly variable clinical spectrum. Main clinical
features include spasticity and hyperreflexia of lower limbs, shuffling gait, mental retardation, aphasia and adducted thumbs. The features of spasticity have been referred to as complicated spastic paraplegia type 1 (SPG1). Some patients manifest corpus callosum hypoplasia and hydrocephalus. Inter- and intrafamilial variability is very wide, such that patients with hydrocephalus, MASA, SPG1, and agenesis of corpus callosum can be present within the same family.

Defects in L1CAM are the cause of spastic paraplegia X-linked type 1 (SPG1) [MIM:303350]. Spastic paraplegia is a degenerative spinal cord disorder characterized by a slow, gradual, progressive weakness and spasticity of the lower limbs.

Note=Defects in L1CAM may contribute to Hirschsprung disease by modifying the effects of Hirschsprung disease-associated genes to cause intestinal aganglionosis.

Defects in L1CAM are a cause of partial agenesis of the corpus callosum (ACCPX) [MIM:304100]. A syndrome characterized by partial corpus callosum agenesis, hypoplasia of inferior vermis and cerebellum, mental retardation, seizures and spasticity. Other features include microcephaly, unusual facies, and Hirschsprung disease in some patients.

Sequence similarities
Belongs to the immunoglobulin superfamily. L1/neurofascin/NgCAM family. Contains 5 fibronectin type-III domains.
Contains 6 Ig-like C2-type (immunoglobulin-like) domains.

Cellular localization
Cell membrane.

Images
ab24345 staining L1CAM in undifferentiated Neuro-2A cells (top panel) and TRA-differentiated Neuro-2A cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1%PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab24345 at 5μg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control, at 1/1000 dilution. Cells were then incubated with ab150117, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (shown in green) and ab150084, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This image was generated using the ascites version of the product.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-L1CAM antibody [2C2] (ab24345)

IHC image of L1CAM staining in a section of formalin-fixed paraffin-embedded normal rat kidney performed on a Leica BOND™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab24345, 0.5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

This image was generated using the ascites version of the product.

Western blot - Anti-L1CAM antibody [2C2] (ab24345)

All lanes:
- **Lane 1**: Mouse whole brain tissue lysate
- **Lane 2**: Rat whole brain tissue lysate
- **Lane 3**: Human whole brain tissue lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Observed band size**: 200 kDa
**Additional bands at**: 60 kDa (possible cleavage fragment)

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 55 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before ab24345 and ab181602 (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at a 1µg/ml concentration and 1/10000
dilution respectively. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution for 1 hour at room temperature before imaging.

This image was generated using the ascites version of the product.

IHC image of L1CAM staining in a section of formalin-fixed paraffin-embedded normal human kidney* performed on a Leica BOND™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab24345, 0.1μg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

This image was generated using the ascites version of the product.

ab24345 staining L1CAM in undifferentiated PC12 cells (top panel) and NGF-differentiated PC12 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1%PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab24345 at 5μg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control, at 1/1000 dilution. Cells were then incubated with ab150117, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (shown in green) and ab150084, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This image was generated using the ascites version of the product.
Anti-L1CAM antibody [2C2] (ab24345) at 1/1000 dilution + 30ug CNS protein

Performed under reducing conditions.

**Observed band size:** 200 kDa

**Additional bands at:** 60-80 kDa (possible cleavage fragment)

ab24345 recognizes one or two polypeptides of L1 or Ng-CAM corresponding to the full length protein (~200kDa) as well as 60-80 kDa C-terminal cleavage products (as shown in the figure).

This image was generated using the ascites version of the product.

Immunofluorescence analysis of COS7 cells transfected with full-length L1CAM (left) or truncated L1CAM (right), staining L1CAM (green) with ab24345.

Cells were incubated with primary antibody (1/1000 in 1% goat serum + 0.3% Triton X-100 in PBS) and incubated overnight at 4°C. An AlexaFluor®488-conjugated anti-mouse IgG (1/700) was used as the secondary antibody. Nuclei were counterstained with bisbenzimide (blue).

This image was generated using the ascites version of the product.

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