abcam

ab316111

Anti-SPG3A/ATL1 antibody [EPR28057-64]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR28057-64
Purification technique	Affinity purification Protein A
Concentration	0.536 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Transfected cell lysate - Human
Dilution info	1/1000
Notes	-

Species	Human

Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/1000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Mouse
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Species	Mouse
Dilution info	1/50
Notes	-

Species	Rat
Dilution info	1/50
Notes	-

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Species	Rat
Dilution info	1/50
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.

Notes	-

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500
Notes	-

Species	Rat
Dilution info	1/500
Notes	-

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Target data

See full target information ATL1

Function	Atlastin-1 (ATL1) is a membrane-anchored GTPase that mediates the GTP-dependent fusion of endoplasmic reticulum (ER) membranes, maintaining the continuous ER network. It facilitates the formation of three-way junctions where ER tubules intersect (PubMed:14506257, PubMed:18270207, PubMed:19665976, PubMed:27619977, PubMed:34817557, PubMed:38509071). Two atlastin-1 on neighboring ER tubules bind GTP and form loose homodimers through the GB1/RHD3-type G domains and 3HB regions. Upon GTP hydrolysis, the 3HB regions tighten, pulling the membranes together to drive their fusion. After fusion, the homodimer disassembles upon release of inorganic phosphate (Pi). Subsequently, GDP dissociates, resetting the monomers to a conformation ready for a new fusion cycle (PubMed:14506257, PubMed:21220294, PubMed:21368113, PubMed:23334294, PubMed:38509071). May also regulate more or less directly Golgi biogenesis (PubMed:17321752). Indirectly regulates axonal development (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	SPG3A also known as Atlastin-1 or ATL1 is a GTPase enzyme with a molecular mass of approximately 64 kDa. This protein is highly important in the mechanical process of membrane trafficking and the maintenance of the endoplasmic reticulum (ER) network. Atlastin-1 is expressed predominantly in neurons especially in the cerebral cortex and the hippocampus where it plays a role in neural function.
Biological function summary	Atlastin-1 is involved in the fusion of ER membranes contributing to the network's tubular structure. The protein facilitates the dimerization and fusion of ER membranes a function that is fundamental for creating a continuous and smooth ER network. While it does not form part of a large protein complex its role in membrane dynamics involves interaction with other proteins that maintain ER structure and function.
Pathways	Atlastin-1 participates in the ER and Golgi-related pathways essential for protein trafficking and cellular homeostasis. It has significant interactions in these pathways with proteins like REEP1 and spastin both of which assist in maintaining the dynamic structure of the ER. These interactions underline Atlastin-1's contribution to vesicle transport and membrane fusion processes.
Associated diseases and disorders	Mutations in the Atlastin-1 gene can lead to hereditary spastic paraplegia (HSP) a group of inherited disorders characterized by weakness and stiffness of the legs. This protein interacts in the context of HSP with proteins like spastin both of which

are implicated in similar neuronal membrane dynamics disorders. Another condition linked to Atlastin-1 dysregulation is neuropathy where the protein's failure to maintain ER integrity can contribute to neuronal degradation.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

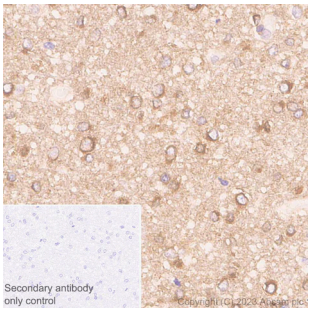
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

24 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

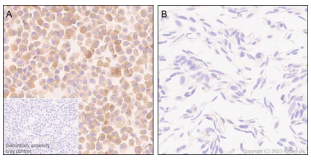
Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling SPG3A/ATL1 with ab316111 at 1/1000 (0.502 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in human cerebrum (PMID: 14506257).
The section was incubated with ab316111 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

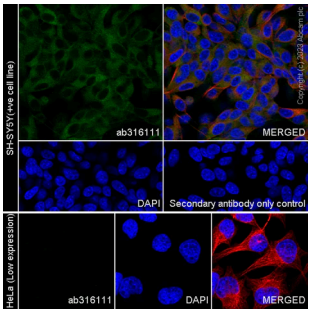
Immunohistochemical analysis of paraffin-embedded A SH-SY5Y (human neuroblastoma epithelial cell) cell pellets; B HeLa (human cervical adenocarcinoma epithelial cell) cell pellets. tissue labeling SPG3A/ATL1 with ab316111 at 1/1000 (0.502 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in SH-SY5Y cell pellets (image A) and weak staining in HeLa cell pellets (image B). The section was incubated with ab316111 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



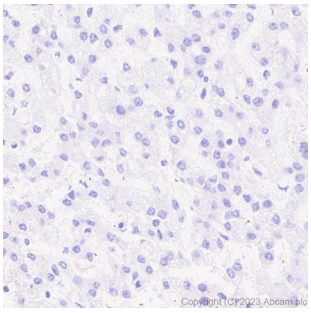
Immunocytochemistry/ Immunofluorescence - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized SH-SY5Y (human neuroblastoma epithelial cell) cells labelling SPG3A/ATL1 with ab316111 at 1/50 (10.04 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/mL) dilution (Green).

Confocal image showing cytoplasmic staining in SH-SY5Y cells.
Low expression: HeLa (PMID: 18270207).
Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human liver using rabbit Anti-SPG3A/ATL1 antibody

Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling SPG3A/ATL1 with ab316111 at 1/1000 (0.502 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

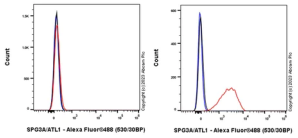
Negative control: No staining in human liver (PMID: 14506257).
The section was incubated with ab316111 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

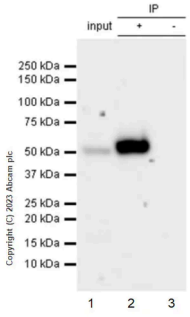


Flow Cytometry (Intracellular) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervical adenocarcinoma epithelial cell, Left) / SH-SY5Y (human neuroblastoma epithelial cell, Right) cells labelling SPG3A/ATL1 with ab316111 at 1/500 dilution (0.1 ug)/Red compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Low expression: HeLa (PMID: 18270207).



Immunoprecipitation - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

SPG3A/ATL1 was immunoprecipitated from 0.35 mg SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate with ab316111 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab316111 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate
Lane 2: ab316111 IP in SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab316111 in SH-SY5Y whole cell lysate.

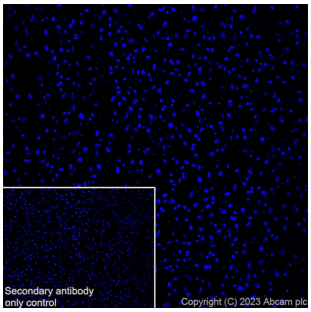
All lanes:
Immunoprecipitation - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111) at 1/30 dilution

All lanes:
SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 24s



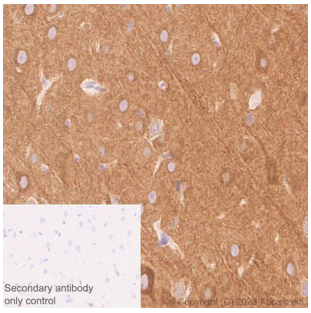
Immunohistochemistry (Frozen sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Immunohistochemistry (Frozen sections) staining of Rat liver (fresh frozen) using rabbit Anti-SPG3A/ATL1 antibody

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat liver (fresh frozen) tissue labeling SPG3A/ATL1 with ab316111 at 1/50 (10.04 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green).

Negative control: confocal image showing no staining on rat liver. The nuclear counterstain was DAPI (Blue). The section was incubated with ab316111 for 60 mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbedat 1/1000 (2 ug/mL) dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling SPG3A/ATL1 with ab316111 at 1/2000 (0.251 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

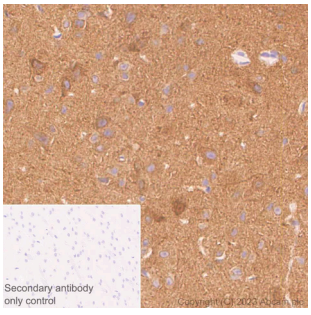
Positive staining in rat cerebrum (PMID: 14506257).
The section was incubated with ab316111 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

---



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

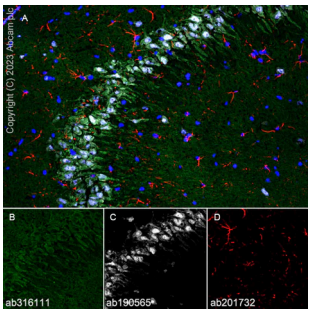
Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling SPG3A/ATL1 with ab316111 at 1/2000 (0.251 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in mouse cerebrum.
The section was incubated with ab316111 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

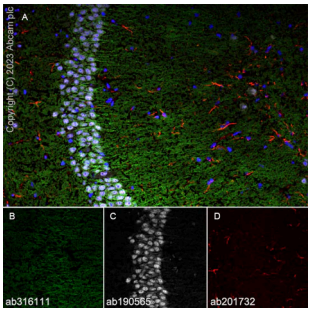


Immunohistochemistry (Frozen sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat hippocmapus (fresh frozen) tissue labeling SPG3A/ATL1 with ab316111 at 1/50 (10.72 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green).

Panel A: merged staining of anti-SPG3A/ATL1 (ab316111, green), anti-NeuN (ab190565, grey) and anti-GFAP (ab201732, red) on rat hippocampus.
Panel B: anti-SPG3A/ATL1 stained on rat hippocampus.
Panel C: anti-NeuN stained in neurons of rat hippocampus.
Panel D: anti-GFAP stained in astrocytes of rat hippocampus.
The nuclear counterstain was DAPI (Blue). The section was incubated with ab316111 fro 60 mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbedat 1/1000 (2 ug/mL) dilution.

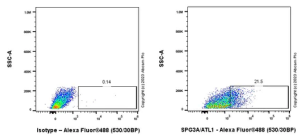


Immunohistochemistry (Frozen sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse hippocmapus (fresh frozen) tissue labeling SPG3A/ATL1 with ab316111 at 1/50 (10.72 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green).

Panel A: merged staining of anti-SPG3A/ATL1 (ab316111, green), anti-NeuN (ab190565, grey) and anti-GFAP (ab201732, red) on mouse hippocampus.
Panel B: anti-SPG3A/ATL1 stained on mouse hippocampus.
Panel C: anti-NeuN stained in neurons of mouse hippocampus.
Panel D: anti-GFAP stained in astrocytes of mouse hippocampus.
The nuclear counterstain was DAPI (Blue). The section was incubated with ab316111 fro 60 mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

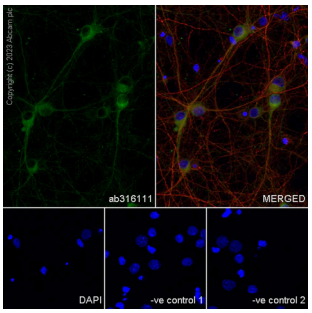
Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbedat 1/1000 (2 ug/mL) dilution.



Flow Cytometry (Intracellular) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Rat primary neuron cells labelling SPG3A/ATL1 with ab316111 at 1/500 dilution (0.1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control.

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

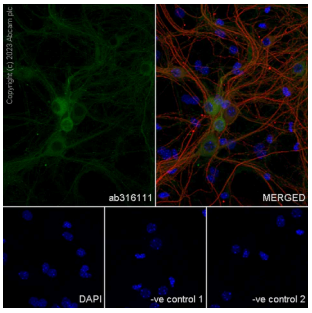
SPG3A/ATL1 Immunocytochemistry/ Immunofluorescence staining of rat primary neural/glia cell using rabbit Anti-SPG3A/ATL1 antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized rat primary neural/glia cell cells labelling SPG3A/ATL1 with ab316111 at 1/50 (10.72 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/mL) dilution (Green).

Confocal image showing cytoplasmic staining in rat primary neuron.
Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.
The image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab11267 Anti-MAP2 mouse monoclonal antibody was used to counterstain at 1/500 (4 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution.



Immunocytochemistry/ Immunofluorescence - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

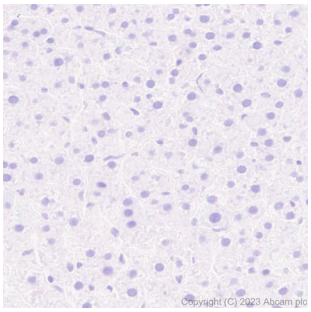
SPG3A/ATL1 Immunocytochemistry/ Immunofluorescence staining of mouse primary neural/glia cell using rabbit Anti-SPG3A/ATL1 antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mouse primary neural/glia cell cells labelling SPG3A/ATL1 with ab316111 at 1/50 (10.72 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/mL) dilution (Green).

Confocal image showing cytoplasmic staining in mouse primary neuron.
Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.
The image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab11267 Anti-MAP2 mouse monoclonal antibody was used to counterstain at 1/500 (4 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat liver using rabbit Anti-SPG3A/ATL1 antibody

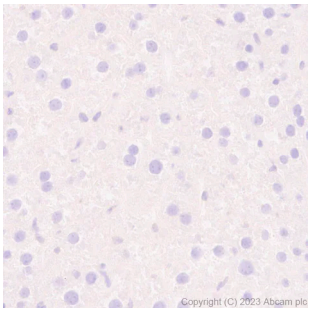
Immunohistochemical analysis of paraffin-embedded Rat liver tissue labeling SPG3A/ATL1 with ab316111 at 1/2000 (0.502 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Negative control: No staining in rat liver.
The section was incubated with ab316111 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse liver using rabbit Anti-SPG3A/ATL1 antibody

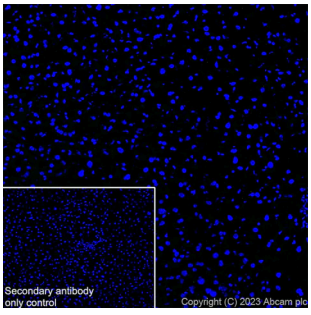
Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling SPG3A/ATL1 with ab316111 at 1/2000 (0.502 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Negative control: No staining in mouse liver.
The section was incubated with ab316111 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



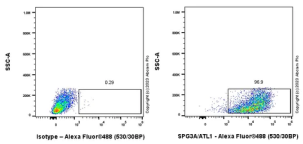
Immunohistochemistry (Frozen sections) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Immunohistochemistry (Frozen sections) staining of Mouse liver (fresh frozen) using rabbit Anti-SPG3A/ATL1 antibody

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse liver (fresh frozen) tissue labeling SPG3A/ATL1 with ab316111 at 1/50 (10.04 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green).

Negative control: confocal image showing no staining on mouse liver. The nuclear counterstain was DAPI (Blue). The section was incubated with ab316111 for 60 mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

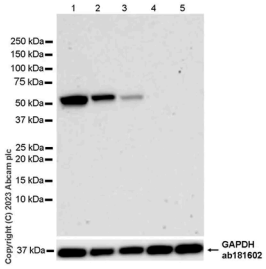
Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbedat 1/1000 (2 ug/mL) dilution.



Flow Cytometry (Intracellular) - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Mouse primary neuron cells labelling SPG3A/ATL1 with ab316111 at 1/500 dilution (0.1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control.

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Western blot staining using rabbit Anti-SPG3A/ATL1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Low expression: HeLa(PMID: 18270207), MCF7.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111) at 1/1000 dilution

Lane 1:
SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
SK-N-BE(2) (human neuroblastoma neuroblast) whole cell lysate at 20 µg

Lane 3:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 4:
MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate at 20 µg

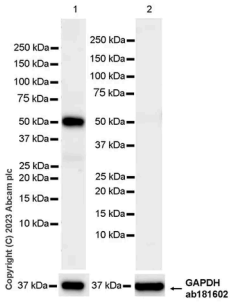
Lane 5:
Hela (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

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

Observed band size: 63 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Western blot staining using rabbit Anti-SPG3A/ATL1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: liver(PMID:14506257)(PMID:18270207).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111) at 1/1000 dilution

Lane 1:
Human brain tissue lysate at 20 µg

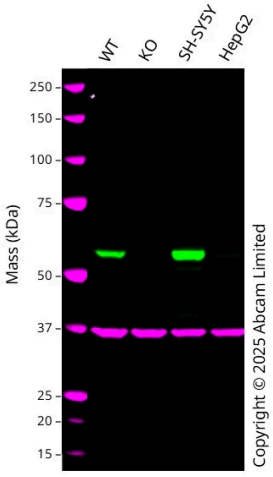
Lane 2:
Human liver tissue lysate at 20 µg

Secondary

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

Observed band size: 63 kDa, 36 kDa

Exposure time: 26s



Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Western blot staining using rabbit Anti-SPG3A/ATL1 antibody

Western blot: Anti-SPG3A/ATL1 antibody [EPR28057-64] ab316111 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH (ab8245) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 64 kDa in Wild-type A549 cell lysates with no signal observed at this size in ATL1 knockout A549 cell line (ab326073). To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) 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 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111) at 1/1000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
Western blot - Human ATL1 knockout A549 cell line (ab326073) at 20 µg

Lane 3:
SH-SY5Y at 20 µg

Lane 4:
HepG2 at 20 µg

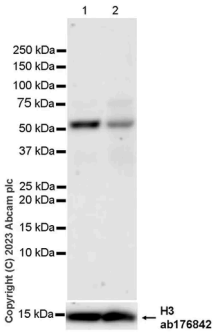
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 64 kDa

Observed band size: 64 kDa



Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Western blot staining using rabbit Anti-SPG3A/ATL1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:
Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) transfected with scrambled siRNA control whole cell lysate at 20 µg

Lane 2:
293T transfected with siRNA specifically targeting ATL1 whole cell lysate at 20 µg

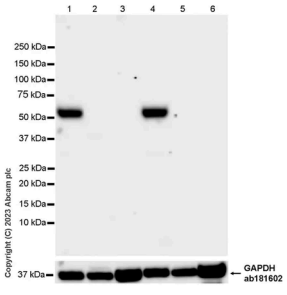
Secondary

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

Developed using the ECL technique.

Observed band size: 63 kDa, 15 kDa

Exposure time: 92s



Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Western blot staining using rabbit Anti-SPG3A/ATL1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: liver, skeletal muscle(PMID: 14506257)(PMID: 18270207).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111) at 1/1000 dilution

Lane 1:
Mouse brain tissue lysate at 20 µg

Lane 2:
Mouse liver tissue lysate at 20 µg

Lane 3:
Mouse skeletal muscle tissue lysate at 20 µg

Lane 4:
Rat brain tissue lysate at 20 µg

Lane 5:
Rat liver tissue lysate at 20 µg

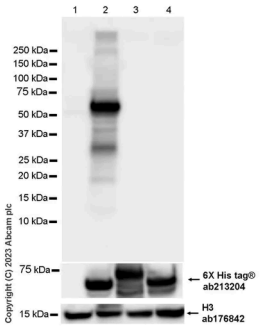
Lane 6:
Rat skeletal muscle tissue lysate at 20 µg

Secondary

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

Observed band size: 63 kDa, 36 kDa

Exposure time: 26s



Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111)

SPG3A/ATL1 Western blot staining using rabbit Anti-SPG3A/ATL1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

This antibody does not cross-react with human ATL2 and ATL3.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

In Western blot, Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204) staining at 1/5000 dilution.

All lanes:
Western blot - Anti-SPG3A/ATL1 antibody [EPR28057-64] (ab316111) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) cells transfected with an empty vector containing a myc-His-tag®, whole cell lysate at 20 µg

Lane 2:
293T cells transfected with a Human ATL1 expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

Lane 3:
293T cells transfected with a Human ATL2 expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

Lane 4:
293T cells transfected with a Human ATL3 expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

Secondary

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

Observed band size: 63 kDa, 15 kDa

Exposure time: 5s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.