abcam

ab300527

Anti-NOTCH3 antibody [EPR25183-101]

Anti-NOTCH3 antibody [EPR25183-101] is a Rabbit Monoclonal antibody that is used in NOTCH3 Flow Cytometry (Intra), IHC-P, Western Blot. Suitable for Human samples.

NOTCH3 is a cell-surface receptor that is widely expressed on vascular smooth-muscle cells. Mutations in the NOTCH3 gene are primarily reponsible for the development of CADASIL syndrome, a rare but devastating cerebrovascular disorder that provides an opportunity to understand the mechanisms underlying other types of small-vessel arteriopathies, vascular dementia, and the relationship between migraine and stroke. Therapeutic antibodies targeting NOTCH3 are being investigated for both CADASIL and a wide range of cancer types.

Recombinant | RabMAb | 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	EPR25183-101
Purification technique	Affinity purification Protein A
Concentration	0.506 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/500
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

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

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50

Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information NOTC3_HUMAN

Function	Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination (PubMed:15350543, PubMed:14714274). Upon ligand activation through the released notch intracellular domain (NICD), it forms a transcriptional activator complex with RBPJ/RBPSUH and activates genes of the enhancer of split locus. Affects the implementation of differentiation, proliferation and apoptotic programs (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

ab300527 does not react in WB with mouse, IHC-P with mouse and rat, ICC with mouse, and intracellular flow cytometry with mouse.

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	The NOTCH3 protein a significant player in the NOTCH signaling pathway is a transmembrane receptor involved in cell differentiation. Sometimes referred to as 'Notch3' this protein has a molecular weight of approximately 244 kDa. NOTCH3 is widely expressed in vascular smooth muscle cells and tissues such as arteries and lungs. The protein also plays an important role in the communication between adjacent cells because of its location on the cell surface.
Biological function summary	NOTCH3 influences various cellular processes including proliferation apoptosis and differentiation. NOTCH3 does not function alone it is part of a regulatory complex that mediates cellular context and fate decisions. The receptor is cleaved upon ligand binding to facilitate intracellular signaling. This signaling is critical in the development and maintenance of vascular integrity which highlights the protein's importance in both physiological and pathological contexts.
Pathways	NOTCH3 plays a role in the NOTCH signaling pathway essential for cell fate determination and the smooth muscle cell differentiation pathway. Ligands such as Jagged and Delta-like proteins trigger NOTCH3 activation. Upon activation the NOTCH3 intracellular domain translocates to the nucleus where it interacts with transcriptional regulators like RBPJ. This interaction modulates the expression of target genes influencing cell differentiation and proliferation.
Associated diseases and disorders	NOTCH3 is implicated in Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) a genetic vascular disorder. Mutations in the NOTCH3 gene result in the accumulation of the NOTCH3 protein within arterial walls leading to the characteristic symptoms of CADASIL. Additionally the protein has been linked to pulmonary arterial hypertension through shared pathways with proteins such as BMP2 and SMADs indicating its broader impact on vascular pathophysiology.

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.

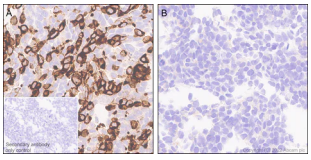
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

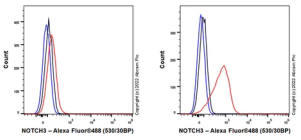
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8 product images



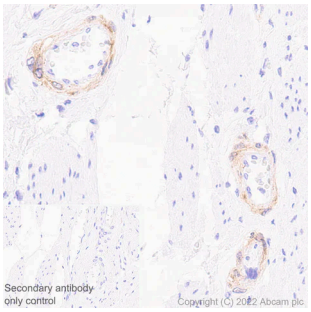
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NOTCH3 antibody [EPR25183-101] (ab300527)

Immunohistochemical analysis of paraffin-embedded Panel A HEK-293T c tissue labeling NOTCH3 with ab300527 at 1/1000 (0.506 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Positive staining on (A) HEK-293T transfected with a human NOTCH3 expression vector, no staining on (B) HEK-293T transfected with empty vector. The section was incubated with ab300527 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) was used.Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



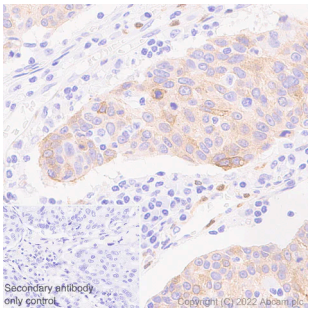
Flow Cytometry (Intracellular) - Anti-NOTCH3 antibody [EPR25183-101] (ab300527)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized MDA-MB-231 (human breast adenocarcinoma epithelial cell, Left) / MCF7 (human breast adenocarcinoma epithelial cell, Right) cells labelling NOTCH3 with ab300527 at 1/50 dilution (1ug) (Red) (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). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Low expression: MDA-MB-231(PMID: 29109797).



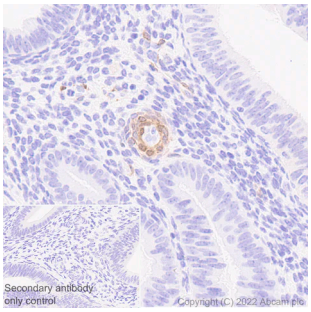
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NOTCH3 antibody [EPR25183-101] (ab300527)

Immunohistochemical analysis of paraffin-embedded Human smooth muscle tissue labeling NOTCH3 with ab300527 at 1/500 (1.012 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Positive staining on vascular smooth muscle cells in human smooth muscle. The section was incubated with ab300527 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) was used.Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



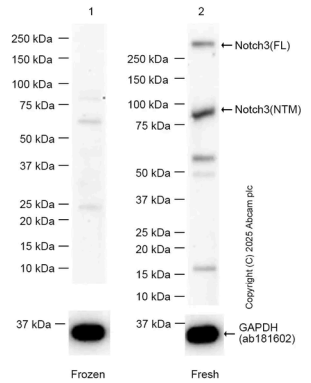
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NOTCH3 antibody [EPR25183-101] (ab300527)

Immunohistochemical analysis of paraffin-embedded Human lung carcinoma tissue labeling NOTCH3 with ab300527 at 1/500 (1.012 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Positive staining on human lung carcinoma. The section was incubated with ab300527 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) was used.Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NOTCH3 antibody [EPR25183-101] (ab300527)

Immunohistochemical analysis of paraffin-embedded Human endometrium tissue labeling NOTCH3 with ab300527 at 1/500 (1.012 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Positive staining on vascular smooth muscle cells of human enodmetrium. The section was incubated with ab300527 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) was used.Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-NOTCH3 antibody [EPR25183-101] (ab300527)

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/1000000 dilution.

Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.

All lanes:
Western blot - Anti-NOTCH3 antibody [EPR25183-101] (ab300527) at 1/1000 dilution

Lane 1:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate (frozen) at 20 µg

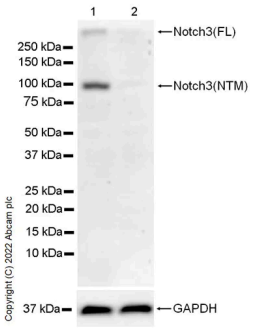
Lane 2:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate (fresh) at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 90 kDa, 270 kDa

Exposure time: 180s



Western blot - Anti-NOTCH3 antibody [EPR25183-101] (ab300527)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

All lanes:
Western blot - Anti-NOTCH3 antibody [EPR25183-101] (ab300527) at 1/1000 dilution

Lane 1:
HeLa (human cervix adenocarcinoma epithelial cell) transfected with scrambled siRNA control, whole cell lysate at 20 µg

Lane 2:
HeLa transfected with siRNA specifically targeti NOTCH3, whole cell lysate at 20 µg

Secondary

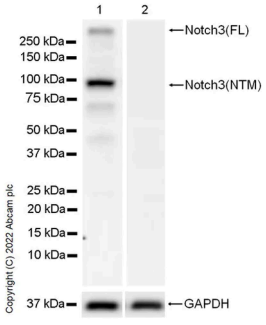
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 90 kDa, 270 kDa

Exposure time: 169s



Western blot - Anti-NOTCH3 antibody [EPR25183-101] (ab300527)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

All lanes:
Western blot - Anti-NOTCH3 antibody [EPR25183-101] (ab300527) at 1/1000 dilution

Lane 1:
MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lane 2:
MDA-MB-231 (human breast adenocarcinoma epithelial cell), whole cell lysate

Secondary

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

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 90 kDa, 270 kDa

Exposure time: 48s

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