abcam

ab246693

Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP1238Y
Purification technique	Affinity purification Protein A
Concentration	1.041 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Endotoxin level is less than 1 EU/ml as determined by the TAL test.

Reactivity data

IHC-P

Tested

Species	Human

Dilution info	-
Notes	-

Not recommended

Species	Mouse, Cow
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Human, Cow
Dilution info	-
Notes	-

WB

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Predicted

Species	Cow
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-

Notes	-

Not recommended

Species	Mouse, Cow
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

Predicted

Species	Cow
Dilution info	-
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Predicted

Species	Cow
Dilution info	-
Notes	-

Target data

See full target information NOTCH1

Function

Functions as a receptor for membrane-bound ligands Jagged-1 (JAG1), Jagged-2 (JAG2) and Delta-1 (DLL1) to regulate cell-fate determination. 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. Involved in angiogenesis; negatively regulates endothelial cell proliferation and migration and angiogenic sprouting. Involved in the maturation of both CD4(+) and CD8(+) cells in the thymus. Important for follicular differentiation and possibly cell fate selection within the follicle. During cerebellar development, functions as a receptor for neuronal DNER and is involved in the differentiation of Bergmann glia. Represses neuronal and myogenic differentiation. May play an essential role in postimplantation development, probably in some aspect of cell specification and/or differentiation. May be involved in mesoderm development, somite formation and neurogenesis. May enhance HIF1A function by sequestering HIF1AN away from HIF1A. Required for the THBS4 function in regulating protective astrogenesis from the subventricular zone (SVZ) niche after injury. Involved in determination of left/right symmetry by modulating the balance between motile and immotile (sensory) cilia at the left-right organiser (LRO).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab246693 is the carrier-free version of ab52627.

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

What does low endotoxin mean?
Our low endotoxin, azide-free formats have low endotoxin level (1 EU/mg, determined by the TAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Supplementary info

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

Activity summary	Notch1 also known as Notch-1 is a transmembrane receptor involved in cell fate decisions. It belongs to the Notch protein family and has a molecular weight of around 270 kDa. Notch1 is expressed widely particularly in tissues such as blood cells and neuronal tissue. The receptor consists of extracellular EGF-like repeats a transmembrane domain and an intracellular domain that translocates to the nucleus upon activation. Notch1 plays a critical role in intercellular communication through ligand engagement which then triggers a series of proteolytic cleavages that release the Notch intracellular domain (NICD) for nuclear translocation.
Biological function summary	Notch1 is essential in various cellular processes including differentiation proliferation and apoptosis. It functions as part of a complex signaling pathway that regulates these processes. Notch1 interacts with other elements like Jagged and Delta/Serrate/LAG-2 (DSL) family ligands to control gene expression patterns that determine cell lineage outcomes. This interaction affects the development of many systems such as the immune and nervous systems. Consequently Notch1 significantly influences the formation of organs and the maintenance of stem cell populations.
Pathways	Notch1 plays a significant role in both the Notch signaling and the Wnt signaling pathways. In the Notch signaling pathway Notch1 upon ligand binding partners with CSL (CBF1/RBP-Jκ in mammals) and Mastermind-like proteins for transcriptional regulation. This pathway interlinks with the Wnt pathway that involves proteins like β-catenin affecting the regulation of gene transcription. The interplay between Notch1 and these pathways is fundamental in determining outcomes in cell proliferation and differentiation emphasizing the interconnected nature of signaling networks.
Associated diseases and disorders	Notch1 is associated with T-cell acute lymphoblastic leukemia and breast cancer. Altered Notch1 signaling often through gain-of-function mutations can drive oncogenesis by disrupting normal cell differentiation and promoting uncontrolled proliferation. In T-cell acute lymphoblastic leukemia aberrant activation of Notch1 leads to increased expression of target genes working closely with related proteins like c-Myc. In breast cancer dysregulation of Notch1 signaling may facilitate tumor growth and metastasis indicating its role as a therapeutic target.

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

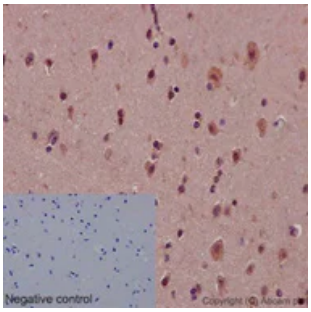
We do not recommend this combination. It is not covered by our product promise.

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.

12 product images

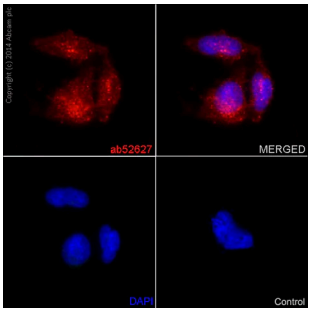


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Immunohistochemical staining of paraffin-embedded human brain with unpurified ab52627 at a dilution of 1/100.
A prediluted HRP polymer for rabbit IgG was used as the secondary and the sample was stained with hematoxylin. PBS was used instead of the primary antibody as the **negative control**, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

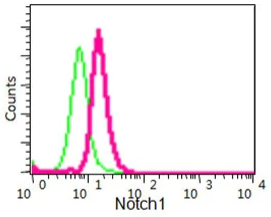


Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Notch1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Notch1 antibody

Immunofluorescent staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells fixed with 4% PFA using purified ab52627 at a dilution of 1/150.
An Alexa Fluor® 555 goat anti-rabbit was used as the secondary and the sample was stained with DAPI. An Alexa Fluor® 555 goat anti-mouse was used at a dilution of 1/500 after ab52627 as the **negative control** and is shown in the bottom right hand panel.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).

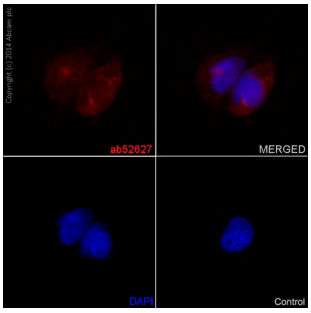


Flow Cytometry (Intracellular) - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Notch1 Flow Cytometry (Intracellular) staining using rabbit Anti-Notch1 antibody

Intracellular Flow Cytometry analysis of permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells (2% PFA, pink) withpurified ab52627 at a 1/200 dilution, or **negative control** rabbit monoclonal IgG (green). The secondary antibody was FITC goat anti-rabbit.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).

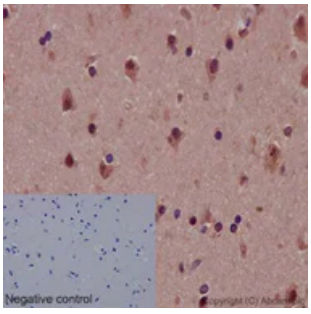


Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Notch1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Notch1 antibody

Immunofluorescent staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells fixed with 4% PFA using unpurified ab52627 at a dilution of 1/100.
An Alexa Fluor® 555 goat anti-rabbit was used as the secondary and the sample was stained with DAPI. An Alexa Fluor® 555 goat anti-mouse was used at a dilution of 1/500 as the **negative control** and is shown in the bottom right hand panel.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).



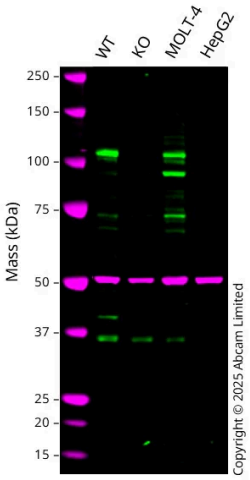
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Notch1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Notch1 antibody

Immunohistochemical staining of paraffin-embedded human brain with purified ab52627 at a dilution of 1/150.
A prediluted HRP polymer for rabbit IgG was used as the secondary and the sample was stained with hematoxylin. PBS was used instead of the primary antibody as the **negative control**, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Notch1 Western blot staining using rabbit Anti-Notch1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).

Western blot: Rabbit Monoclonal [EP1238Y] to Notch1 ab52627 staining at 1/1000 dilution, shown in green; Mouse anti alpha Tubulin ab7291 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 105 kDa in Wild-type HCT 116 cell lysates with no signal observed at this size in NOTCH1 knockout HCT 116 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5pc 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 and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:

Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 whole cell lysate at 20 µg

Lane 2:
Western blot - Human NOTCH1 knockout HCT116 cell line (ab287652) at 20 µg

Lane 3:
MOLT-4 whole cell lysate at 20 µg

Lane 4:
HepG2 whole cell lysate at 20 µg

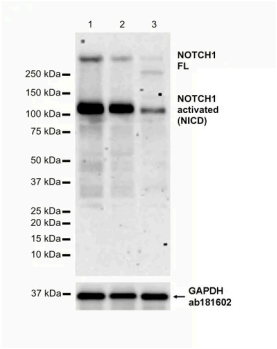
Secondary

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

Performed under reducing conditions.

Predicted band size: 100 kDa

Observed band size: 105 kDa



Western blot - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Notch1 Western blot staining using rabbit Anti-Notch1 antibody

This data was developed using ab52627, the same antibody clone in a different buffer formulation. Blocking and diluting buffer and concentration: 5% NFDM/TBST.

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

All lanes:
Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627) at 1/1000 dilution

Lane 1:
HeLa (human cervical adenocarcinoma epithelial cell)

Lane 2:
293T (human embryonic kidney epithelial cell)

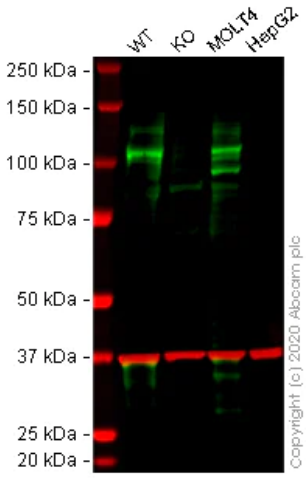
Lane 3:
NIH/3T3 (mouse embryonic fibroblast)

Secondary

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

Observed band size: 110 kDa, 272 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Notch1 Western blot staining using rabbit Anti-Notch1 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab52627).
Lanes 1 - 4: Merged signal (red and green). Green - ab52627 observed at 110-120 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab52627 was shown to react with Notch1 in wild-type HAP1 cells in western blot. Loss of signal was observed when NOTCH1 knockout sample was used. Wild-type and NOTCH1 knockout HAP1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3pc milk before incubation with ab52627 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627) at 1 µg/mL

Lane 1:
Wild-type HAP1 cell lysate at 40 µg

Lane 2:
NOTCH1 knockout HAP1 cell lysate at 40 µg

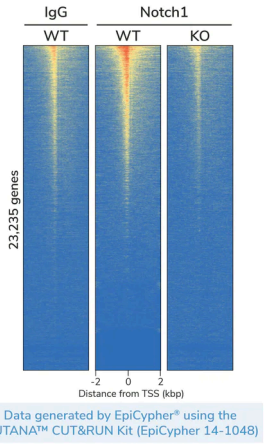
Lane 3:
MOLT-4 cell lysate at 20 µg

Lane 4:
HepG2 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 272 kDa

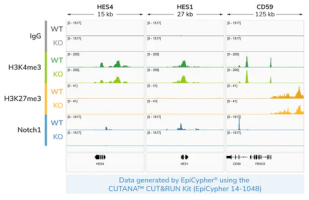
Observed band size: 110-120 kDa



ChIC/CUT&RUN sequencing - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).

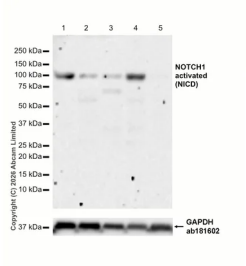
CUT&RUN profiling with Notch1 antibody demonstrates robust genome-wide enrichment in wild-type (WT) cells, which is markedly diminished in NOTCH1 knockout (KO) cells. Heatmaps of genome-wide signal flanking annotated transcription start sites (TSSs, +/- 2 kbp) display CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with Notch1 antibody (Abcam ab52627, 0.5 µg). 500,000 HeLa WT or KO (Abcam ab261762) cells were used per reaction. IgG antibody was included as a negative control to assess non-specific background. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Heatmaps were generated using deepTools (Ramнrez et al., Nucleic Acids Res. 2014; PMID: 24799436). Row-linked data are ranked by intensity relative to Notch1 WT, with red indicating high localized enrichment and blue denoting background. Validated antibodies show genome-wide enrichment above IgG background consistent with Notch1 binding in WT cells and near complete loss of signal in KO cells.



ChIC/CUT&RUN sequencing - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).

CUT&RUN profiling with Notch1 antibody reveals the expected genomic enrichment pattern in wild-type (WT) cells, which is substantially reduced in NOTCH1 knockout (KO) cells. Representative genome browser tracks show CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with Notch1 antibody (Abcam ab52627, 0.5 µg). 500,000 HeLa WT or KO (ab261762) cells were used per reaction. IgG, H3K4me3, and H3K27me3 antibodies were included as controls to assess non-specific background, active promoters, and repressed chromatin, respectively. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Images were generated using Integrative Genomics Viewer (IGV, Broad Institute).



Western blot - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Notch1 Western blot staining using rabbit Anti-Notch1 antibody

This data was developed using ab52627, the same antibody clone in a different buffer formulation. Blocking and diluting buffer and concentration: 5% NFDM/TBST.

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

Low expression: Ovary.

All lanes:
Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627) at 1/1000 dilution

Lane 1:
Mouse brain tissue lysate at 40 µg

Lane 2:
Mouse heart tissue lysate at 40 µg

Lane 3:
Mouse spleen tissue lysate at 40 µg

Lane 4:
Mouse lung tissue lysate at 40 µg

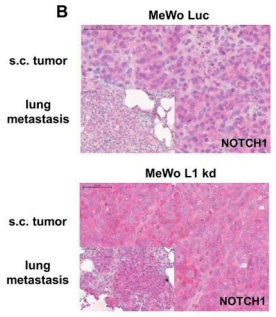
Lane 5:
Mouse ovary tissue lysate at 40 µg

Secondary

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

Observed band size: 110 kDa, 36 kDa

Exposure time: 180s



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

Verification of gene expression array data by immunohistochemical analysis of Notch 1 expression in subcutaneous tumors and lung metastases from a human melanoma (MeWo) xenograft experiment in mice.
Immunohistochemical staining for Notch1 expression (ab52627, red) in subcutaneous tumors and lung metastases (both panels) of MeWo (Human malignant melanoma cell line) cells.

All scale bars: 50 µm.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52627).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

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