abcam

Product datasheet

Anti-Notch1 antibody [EP1238Y] - BSA and Azide free ab221603

KO VALIDATED Recombinant RabMAb

1 References 9 Images

Overview

Product name	Anti-Notch1 antibody [EP1238Y] - BSA and Azide free
Description	Rabbit monoclonal [EP1238Y] to Notch1 - BSA and Azide free
Host species	Rabbit
Specificity	80% identities with Notch 2 and 81% with Notch 3
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt (Intra) **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Cow
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK293, HAP1 and MOLT-4 cell lysate. IHC-P: Human brain tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells.
General notes	ab221603 is the carrier-free version of **ab52627**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1238Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab221603 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 at an assay dependent concentration. Detects a band of approximately 125 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Application notes Is unsuitable for IP.

Target

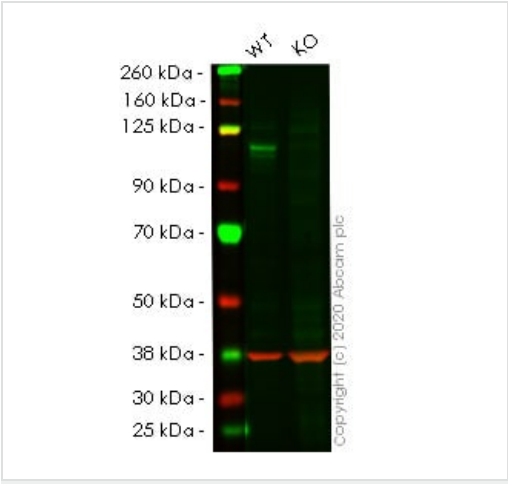
Function Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination. Upon ligand activation through the released notch intracellular domain (NICD) it forms a transcriptional activator complex with RBPJ/RBPSUH and activates genes of the enhancer of split locus. Affects the implementation of differentiation, proliferation and apoptotic programs. May be important for normal lymphocyte function. In altered form, may contribute to transformation or progression in some T-cell neoplasms. Involved in the maturation of both CD4+ and CD8+ cells in the thymus. May be important for follicular differentiation and possibly cell fate selection within the follicle. During cerebellar development, may function as a receptor for neuronal DNER and may be involved in the differentiation of Bergmann glia.

Tissue specificity In fetal tissues most abundant in spleen, brain stem and lung. Also present in most adult tissues where it is found mainly in lymphoid tissues.

Involvement in disease Defects in NOTCH1 are a cause of bicuspid aortic valve (BAV) [MIM:109730]. A common defect

	in the aortic valve in which two rather than three leaflets are present. It is often associated with aortic valve calcification and insufficiency. In extreme cases, the blood flow may be so restricted that the left ventricle fails to grow, resulting in hypoplastic left heart syndrome.
Sequence similarities	Belongs to the NOTCH family. Contains 5 ANK repeats. Contains 36 EGF-like domains. Contains 3 LNR (Lin/Notch) repeats.
Post-translational modifications	Synthesized in the endoplasmic reticulum as an inactive form which is proteolytically cleaved by a furin-like convertase in the trans-Golgi network before it reaches the plasma membrane to yield an active, ligand-accessible form. Cleavage results in a C-terminal fragment N(TM) and a N-terminal fragment N(EC). Following ligand binding, it is cleaved by TNF-alpha converting enzyme (TACE) to yield a membrane-associated intermediate fragment called notch extracellular truncation (NEXT). This fragment is then cleaved by presenilin dependent gamma-secretase to release a notch-derived peptide containing the intracellular domain (NICD) from the membrane. Phosphorylated. O-glycosylated on the EGF-like domains. Contains both O-linked fucose and O-linked glucose. Ubiquitinated; undergoes 'Lys-29'-linked polyubiquitination catalyzed by ITCH.
Cellular localization	Cell membrane and Nucleus. Following proteolytical processing NICD is translocated to the nucleus.

Images



Western blot - Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

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

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : NOTCH1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

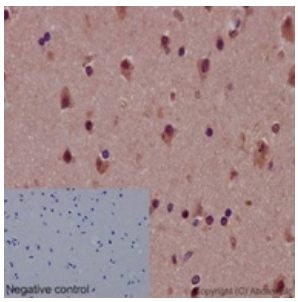
Observed band size: 110 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab52627**).

Lanes 1- 2: Merged signal (red and green). Green - **ab52627** observed at 110 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab52627 was shown to react with Notch1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab261762** (knockout cell lysate **ab257006**) was used. Wild-type HeLa and NOTCH1 knockout HeLa cell lysates were subjected to

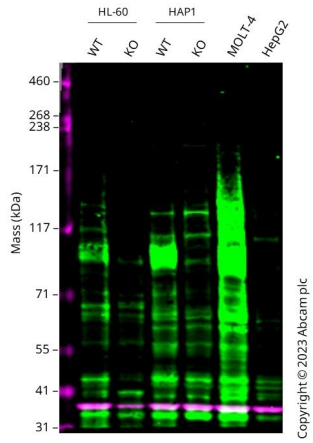
SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab52627** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

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**).



Western blot - Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

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

Lane 1 : Wild-type HL-60 cell lysate
Lane 2 : NOTCH1 knockout HL-60 cell lysate
Lane 3 : Wild-type HAP1 cell lysate
Lane 4 : NOTCH1 knockout HAP1 cell lysate
Lane 5 : MOLT-4 cell lysate
Lane 6 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Developed using the ECL technique.

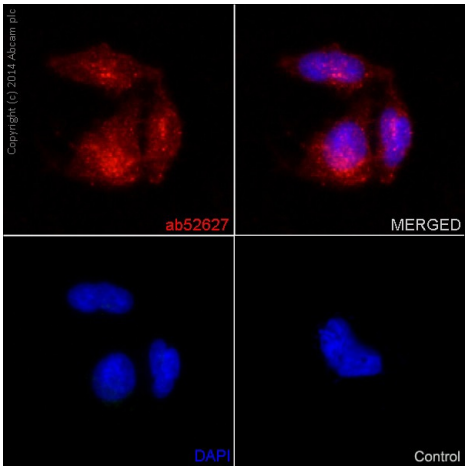
Performed under reducing conditions.

Observed band size: 100 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (**ab52627**).

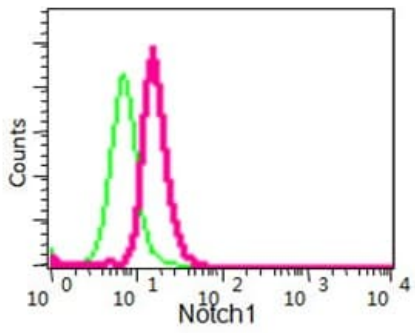
Western blot: Anti-NOTCH1 antibody [EP1238Y] (**ab52627**) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, **ab52627** was shown to bind specifically to NOTCH1. A band was observed at 100 kDa in wild-type HL-60 cell lysates with no signal observed at this size in NOTCH1 knockout cell line. To generate this image, wild-type and NOTCH1 knockout HL-60 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% 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 IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

Immunofluorescent staining of HeLa 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.

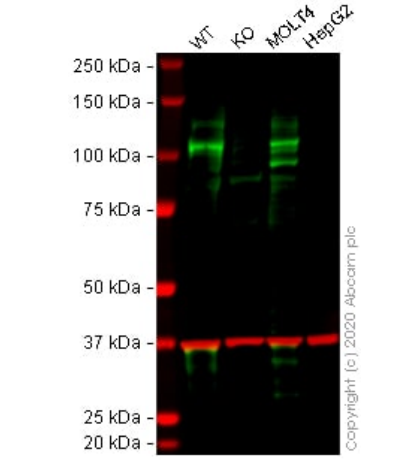
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52627**).



Flow Cytometry (Intracellular) - Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

Intracellular Flow Cytometry analysis of permeabilized HeLa cells (2% PFA, pink) with purified **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**).



Western blot - Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

All lanes : 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

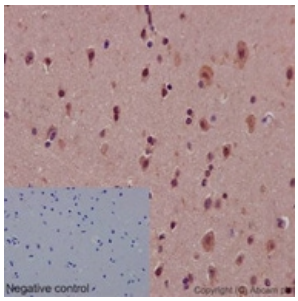
Performed under reducing conditions.

Observed band size: 105 kDa

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 105 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

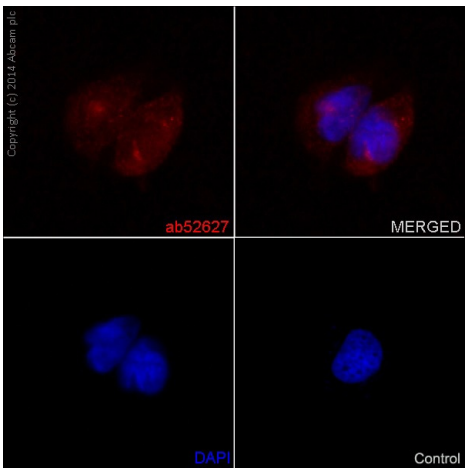
ab52627 was shown to react with Notch1 in wild-type HAP1 cells in western blot. Loss of signal was observed when NOTCH1 knockout sample was used. Wild-type and NOTCH1 knockout HAP1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk before incubation with **ab52627** and **ab8245** (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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

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**).



Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

Immunofluorescent staining of HeLa 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**).

Anti-Notch1 antibody [EP1238Y] - BSA and Azide free (ab221603)

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