abcam

Product datasheet

Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free ab246693

KO VALIDATED Recombinant RabMAb

9 Images

Overview

Product name	Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EP1238Y] to Notch1 - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-P, WB, 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: HEK293, HAP1 and MOLT-4 cell lysateS. IHC-P: Human brain tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells.
General notes	ab246693 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. 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 production For more information **see here**.

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

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

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
Purification notes	Endotoxin level is less than 1 EU/ml as determined by the TAL test.
Clonality	Monoclonal
Clone number	EP1238Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab246693 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
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
WB		Use at an assay dependent concentration. Predicted molecular weight: 272 kDa.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

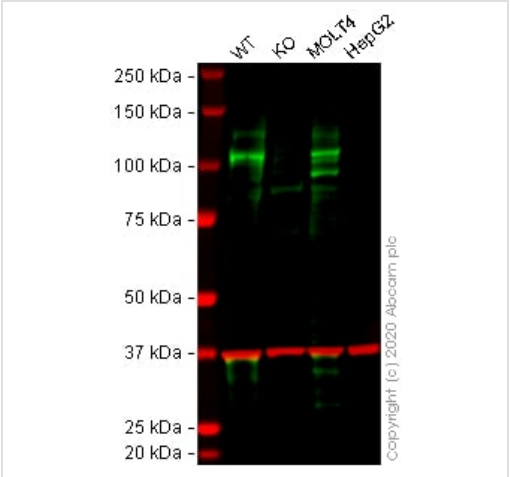
Application notes Is unsuitable for IP.

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] - Low endotoxin, Azide free (ab246693)

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.

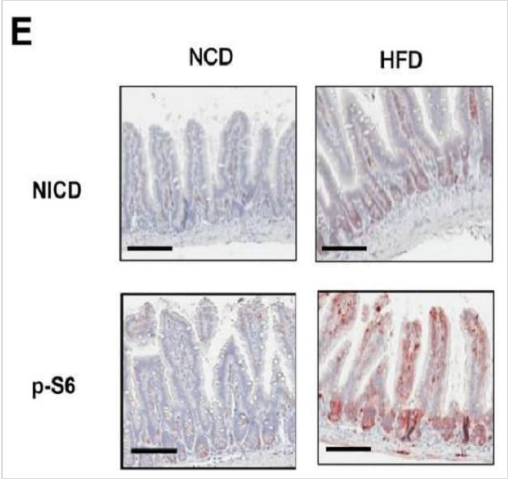
Predicted band size: 272 kDa
Observed band size: 105 kDa

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 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.



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

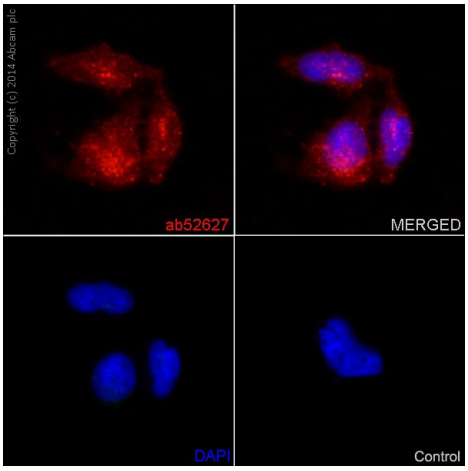
Decrease of goblet cells in mice fed a high-fat diet (HFD).

Immunohistochemistry using anti-Notch intracellular domain (NICD) **ab52627** and phospho-S6 Abs.

Mice intestines were flushed with phosphate-buffered saline (PBS) and fixed in 10% neural formalin overnight at room temperature. The paraffin-embedded specimens were cut into 5 µm sections and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS)/Alcian blue. Paneth cells were stained with purple, and goblet cells blue with the PAS/Alcian blue method.

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.

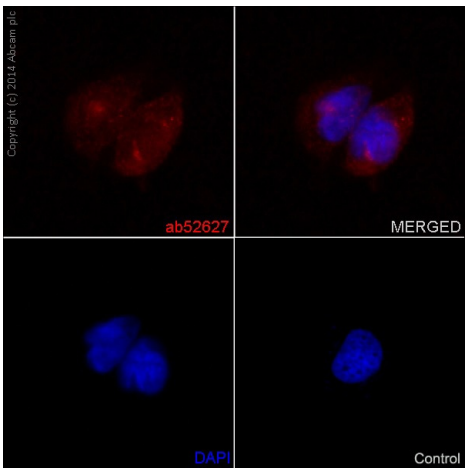


Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

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**).

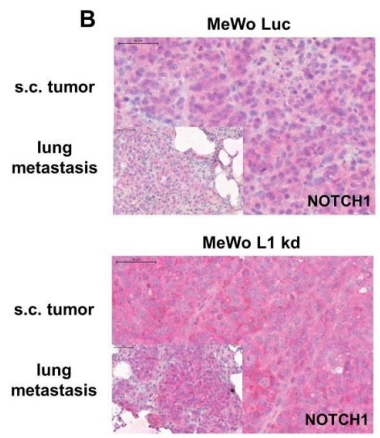


Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

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)

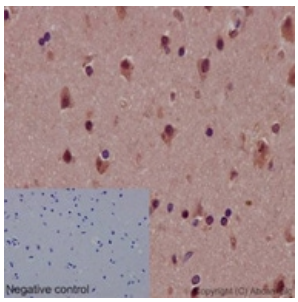
Verification of gene expression array data by immunohistochemical analysis of Notch 1 expression in subcutaneous tumors and lung metastases from a human melanoma (MeWo) xenograft experiment in mice.

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.



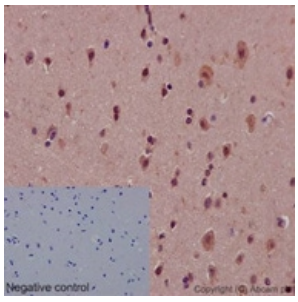
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

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.



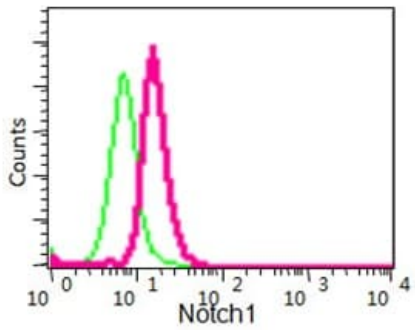
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Notch1 antibody [EP1238Y] - Low endotoxin, Azide free (ab246693)

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.



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

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**).

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

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