Product datasheet

Anti-Notch1 antibody [EP1238Y] ab52627

Overview

Product name  Anti-Notch1 antibody [EP1238Y]
Description  Rabbit monoclonal [EP1238Y] to Notch1
Host species  Rabbit
Specificity  80% identities with Notch 2 and 81% with Notch 3
Tested applications  Suitable for: IHC-P, Flow Cyt, WB, ICC/IF
Unsuitable for: IP
Species reactivity  Reacts with: Mouse, Cow, Human
Immunogen  Synthetic peptide within Human Notch1 aa 2500-2600. The exact sequence is proprietary.
General notes  A trial size is available to purchase for this antibody.

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

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties

Form  Liquid
Storage instructions  Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer  pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity  Protein A purified
Clonality: Monoclonal
Clone number: EP1238Y
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab52627 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IHC-P</td>
<td>🌟🌟🌟🌟</td>
<td>1/100 - 1/150. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/130 - 1/200. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>ICC/IF</td>
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<td>1/100 - 1/150.</td>
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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**

**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.

**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.
Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627)

Anti-Notch1 antibody [EP1238Y] (ab52627) at 1/2000 dilution (Purified) + Mouse brain at 10 µg

Secondary
HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Observed band size:** 125 kDa

*why is the actual band size different from the predicted?*

**Blocking/Dilution buffer:** 5% NFDM/TBST.

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.

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.
Flow cytometry analysis of permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) 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.

Anti-Notch1 antibody [EP1238Y] (ab52627) at 1/2000 dilution (Purified) + HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate at 10 µg

Secondary
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Observed band size: 125 kDa why is the actual band size different from the predicted?

Blocking/Dilution buffer: 5% NFDM/TBST.

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.
Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] (ab52627)

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.

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