Anti-activated Notch1 antibody ab52301

Overview

Product name
Anti-activated Notch1 antibody

Description
Rabbit polyclonal to activated Notch1

Host species
Rabbit

Specificity
ab52301 detects endogenous levels of fragment of activated Notch 1 resulting from cleavage adjacent to Val1744.

Tested applications
Suitable for: IHC-Fr, ICC/IF, IHC-P, WB, ELISA

Species reactivity
Reacts with: Mouse, Rat, Human

Immunogen
Synthetic peptide corresponding to Human activated Notch1 aa 1720-1770.

Sequence:
A VQSETVEPPP PAQLHFMYVA AAAFVLLFFV GC GVLLSRKR RRQHGQLWFP

Database link: P46531

Positive control
Extracts from NIH 3T3 cells, treated with etoposide (25µM, 1hour). IHC-P: Human brain tissue

General notes
ab52301 is cleaved at Val 1744

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: 7.40
Preservative: 0.02% Sodium azide
Constituents: 50% Glycerol, 0.87% Sodium chloride, PBS

Purity
Immunogen affinity purified

Purification notes
ab52301 was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific immunogen.

Clonality
Polyclonal
Isotype

IgG

Applications

Our Abpromise guarantee covers the use of ab52301 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<th>Application</th>
<th>Abreviews</th>
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<td>IHC-Fr</td>
<td>Use at an assay dependent concentration. PubMed: 24098462</td>
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<td>ICC/IF</td>
<td>Use at an assay dependent concentration. PubMed: 20680961</td>
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<tr>
<td>IHC-P</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td>1/500 - 1/1000. Detects a band of approximately 95 kDa (predicted molecular weight: 272 kDa).</td>
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<td>ELISA</td>
<td>1/10000.</td>
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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. Represses neuronal and myogenic differentiation. May enhance HIF1A function by sequestering HIF1AN away from HIF1A.

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 aortic valve disease 1 (AOVD1) [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). Following endocytosis, 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. Monoubiquitination at Lys-1759 is required for activation by gamma-secretase cleavage, it promotes interaction with AAK1, which stabilizes it. Deubiquitination by EIF3F is necessary for nuclear import of activated Notch.

Hydroxylated at Asn-1955 by HIF1AN. Hydroxylated at Asn-2022 by HIF1AN (By similarity).

Hydroxylation reduces affinity for HIF1AN and may thus indirectly modulate negative regulation of NICD.

**Cellular localization**

Cell membrane and Nucleus. Following proteolytical processing NICD is translocated to the nucleus.

**Images**

Immunofluorescence analysis of frozen tissue sections (10µm) of intestine from sham operated rats (control group) or rats after Ischemic-Reperfusion (IR) injury labeling Notch1 with ab52301.

Tissue sections were frozen after being fixed with 4% formaldehyde, then incubated in 3% H₂O₂ for 30 minutes and then blocked with 5% BSA for 30 mins at room temperature. The sections were incubated overnight with ab52301 (PBS + 3% BSA). An appropriate secondary antibody was then added. DAPI (in blue) was used to stain the nucleus.

**All lanes**: Anti-activated Notch1 antibody (ab52301) at 1/500 dilution

**Lane 1**: NIH 3T3 cell extract treated with etoposide (25µM, 1 hour)

**Lane 2**: NIH 3T3 cell extract treated with etoposide (25µM, 1 hour) with immunising peptide

**Predicted band size**: 272 kDa

**Observed band size**: 95 kDa

**why is the actual band size different from the predicted?**
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-activated Notch1 antibody (ab52301)

Immunohistochemical analysis of paraffin-embedded human brain tissue sections labeling activated Notch1 with ab52301 at a dilution of 1/100. The picture on the right is treated with the synthesized peptide.

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