Product datasheet

Anti-activated Notch1 antibody ab8925

Overview

Product name
Anti-activated Notch1 antibody

Description
Rabbit polyclonal to activated Notch1

Host species
Rabbit

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

Species reactivity
Reacts with: Mouse, Human

Predicted to work with: Rat

Immunogen
Synthetic peptide corresponding to activated Notch1 aa 1755-1767 (intracellular). The epitope is only exposed after gamma secretase cleavage and is not accessible in the uncleaved form.

Sequence:
VLLSRKRRQHGQC

(Peptide available as ab730)

Positive control
WB: Lysates from myc-tagged transiently transfected mouse Notch constructs in HEK-293 cells. IHC-Fr: Mouse dermis. IHC-P: Human ovarian carcinoma tissue. ICC/IF: NIH/3T3 cells.

General notes
Notch is synthesized in the endoplasmic reticulum as an inactive form which is proteolytically cleaved by a furin-like convertase (S1 cleavage) 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 (S2 cleavage) 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 (S3 cleavage) to release the intracellular domain (NICD) from the membrane.

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 or -80°C. Avoid freeze / thaw cycle.

Storage buffer
Preservative: 0.1% Sodium azide
Constituents: 0.4% Potassium phosphate, 0.87% Sodium chloride

Purity
Whole antiserum
Purification notes
Label may show concentration. This is total protein concentration. Total and specific IgG concentration have not been determined.

Primary antibody notes
Notch is synthesized in the endoplasmic reticulum as an inactive form which is proteolytically cleaved by a furin-like convertase (S1 cleavage) 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 (S2 cleavage) 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 (S3 cleavage) to release the intracellular domain (NICD) from the membrane.

Clonality
Polyclonal

Isotype
IgG

Applications
Our Abpromise guarantee covers the use of ab8925 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>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-P</td>
<td></td>
<td>1/200.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 18371421</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>1/500. Detects a band of approximately 80 kDa. Can be blocked with Human activated Notch1 peptide (ab730). 80kDa band is a cleavage product of Notch 1. There is also a contaminating band present at 100 kDa. The identity of this band is unknown.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 19171875. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
</tbody>
</table>

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

**The Notch signaling pathway is activated in the crypts.**

**(Panel B)** Immunofluorescence staining for NICD (red, ab8925), with β-catenin in green and DAPI in blue.

For immunofluorescent staining of mouse small intestine cryosections, we used a blocking solution of 1% BSA and 0.1% Triton X-100 in PBS.
TGFβ/Smad transcriptional inhibitors following acute resistance exercise.

The levels of the Smad inhibitors SKI and active Notch were determined at the end of a 20 minute bout of high force lengthening contractions and then 0.5, 3, 6, 18, and 48 hours later. SKI and Notch protein was normalized to GAPDH.

Rat muscles were powdered on dry ice using a mortar and pestle and polytron homogenized in 10-fold mass excess of ice cold sucrose lysis buffer and 0.1% DTT. This was vortexed for 30 minutes at 4°C and centrifuged at 4°C for 10 minutes at 10,000×g to remove insoluble material.

Equal aliquots of protein were diluted in Laemmli sample buffer and boiled for 5 minutes. 5–10 µg of sample was then subjected to SDS-PAGE on 10% acrylamide gels at a constant current equal to 20 mA per gel and transferred to Protran nitrocellulose membrane using a semidry transfer apparatus at 100 V for 1 hour. Membranes were blocked in 5% dry milk in TBST, and then incubated over night at 4°C with ab8925 in TBST at 1:1,000.

The membranes were then washed 3x in TBST before incubation for 1 hour at room temperature with peroxidase-conjugated secondary antibodies in TBST at 1:10,000. ECL detection.

Expression of the Notch-dependent regulatory axes’ components during late retinal histogenesis and in enriched Müller glia (MG) cells from rat.

Briefly, 4% paraformaldehyde-fixed cells or explant cryosections (12 µm thickness) were blocked in blocking solution, followed by an overnight incubation with specific antibodies including ab8925 (shown in image).
ab8925 staining activated Notch 1 in murine dermis (including follicle) by Immunohistochemistry (Frozen sections).

Tissue was fixed with paraformaldehyde and permeabilized using 0.1% Triton. Samples were then blocked with 10% serum for 1 hour at 19°C followed by incubation with the primary antibody at a 1/100 dilution for 16 hours at 4°C. An Alexa Fluor® conjugated donkey anti-rabbit polyclonal was used as secondary antibody at a 1/200 dilution.

Counterstain DAPI (blue).

ab8925 at a 1:500 dilution staining activated Notch1 in human ovarian carcinoma using an automated system (DAKO Autostainer Plus).

Using this protocol there is strong staining of activated Notch 1 in nuclear/nucleolar compartments of the ovarian cortex.

Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH 6.1 in a DAKO PT link. Slides were blocked in 3% H₂O₂/methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes.

Slides were counterstained with hematoxylin and coverslipped under DePeX.

Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.
Western blot - Anti-activated Notch1 antibody
(ab8925)
This image is courtesy of Stacey Huppert, Ma. Xenia U. Garcia (lab of Raphael Kopan)

Rabbit polyclonal (ab8925) at 1/500, against myc-tagged transiently transfected mouse Notch constructs in HEK-293 (Human epithelial cell line from embryonic kidney) cells.

Lane M: Mol wt markers
Lane 1 No transfection
Lane 2 N1 (mouse deleted extracellular domain)-myc
Lane 3 N1 (mouse intracellular domain)-myc
Lane 4 N2 (mouse deleted extracellular domain)-myc
Lane 5 N2 (mouse intracellular domain)-myc
Lane 6 N3 (mouse deleted extracellular domain)-myc
Lane 7 N3 (mouse intracellular domain)-myc
Lane 8 N4 (mouse deleted extracellular domain)-myc
Lane 9 N4 (mouse intracellular domain)-myc
Lane 10 N1 (mouse deleted extracellular domain)(V to G)-myc

ab8925 staining activated Notch1 in the NIH/3T3 (Mouse embryo fibroblast cell line) cells by ICC/IF (Immunocytochemistry/immunofluorescence).

Cells were fixed with paraformaldehyde, permeabilized with Triton X-100 0.1% and blocked with 10% serum for 30 minutes at 24°C. Samples were incubated with primary antibody (1/100) for 16 hours at 4°C. An Alexa Fluor®488-conjugated Donkey anti-rabbit IgG polyclonal(1/500) was used as the secondary antibody.

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