

Datasheet for 100-401-406**NOTCH 2 Antibody****Overview**

Description:	Anti-NOTCH 2 (intra) (Human specific) (RABBIT) Antibody - 100-401-406
Item No.:	100-401-406
Size:	200 µL
Applications:	ELISA, IHC, WB, IF, Multiplex
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Anti Notch 2 Antibody recognizes Notch 2 that 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. Notch 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 RBP-J kappa and activates genes of the enhancer of split locus. Affects the implementation of differentiation, proliferation and apoptotic programs.
Synonyms:	rabbit anti-Notch 2 antibody, AGS 2 antibody, AGS2 antibody, hN2 antibody, N2 antibody, Neurogenic locus notch homolog protein 2 antibody, Notch 2 intracellular domain antibody, Notch homolog 2 antibody, NICD
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	NOTCH2
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Reactivity:	Human
Immunogen Type:	Conjugated Peptide
Immunogen:	This whole rabbit serum was prepared by repeated immunizations with a synthetic peptide corresponding to an internal region near amino acid residues 2390-2415 of human Notch 2 (the total protein is 2471 aa). A residue of cysteine was added to the amino terminal end to facilitate coupling.
Purity/Specificity:	This antiserum is directed against human NOTCH 2. The peptide sequence shows 100% alignment with human, dog and chimpanzee sequence. Only one (1) amino acid difference is found in mouse and this change is non-conservative. Based on the sequence we expect this antibody to react as well with rat and mouse NOTCH 2. No specific information is available for other reactivities.
Relevant Links:	• UniProtKB - Q04721 • NCBI - 24041035 • GeneID - 4853

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF, Multiplex (Based on references)
Application Note:	This antibody has been tested for use in ELISA and western blot. For western blot experiments, one can expect a band of ≈110 kDa in size corresponding to active Notch 2 in the appropriate cell lysate. A 1:500 dilution was effective for staining FFPE human kidney tissue by Immunohistochemistry (IHC). Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:30,000 - 1:90,000
IHC:	1:200-1:800
WB:	1:400 - 1:2,000

Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	75 mg/mL by Refractometry
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.1% (w/v) Sodium Azide

Stabilizer: None

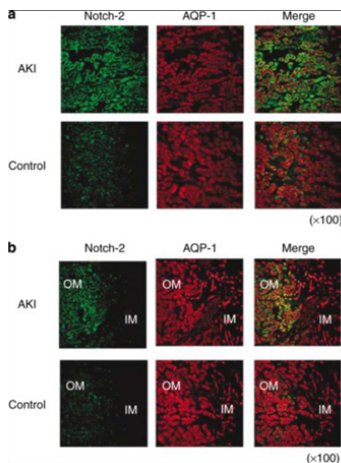
Shipping & Handling

Shipping Condition: Dry Ice

Storage Condition: Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

Expiration: Expiration date is one (1) year from date of receipt.

Images



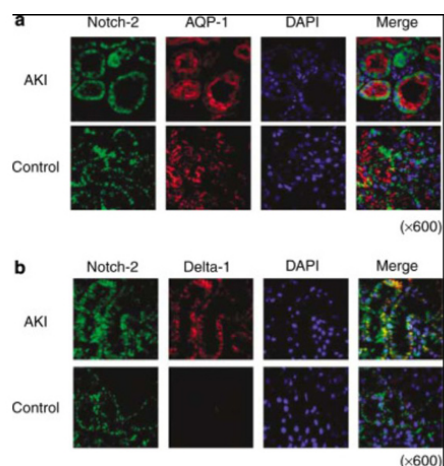
Immunohistochemistry

Immunohistochemical examination of cleaved Notch-2 in the renal cortex of ischemic/reperfused kidneys. (a)

Immunohistochemical analyses of the renal cortex were performed at a low-power view (original magnification, × 100) by using antibodies against Notch-2 and aquaporin-1 (AQP-1) and in a merged condition at 12 h after ischemic injury (upper panels). The corresponding analyses carried out in control kidneys are shown in the lower panels. (b)

Immunohistochemical analyses of the renal cortex performed at a low-power view (original magnification, × 100) using antibodies against Notch-2 and AQP-1 and in a merged condition at 12 h after ischemic injury were carried out in AKI kidneys (upper panels) and in control kidneys (lower panels). IM, inner medulla; Merge, merged condition; OM, outer medulla.

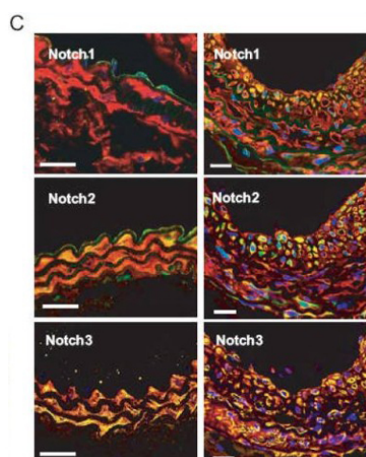
Figure 5. PMID: 18418349.



Immunohistochemistry

Immunohistochemical examination (high-power views) of Notch-2 in the renal outer medulla of ischemic/reperfused kidneys. (a) Immunohistochemical analyses of the renal outer medulla were performed at a high-power view (original magnification, $\times 600$) by using antibodies against Notch-2 aquaporin-1 (AQP-1), and 4',6-diamidino-2-phenylindole (DAPI) and in a merged condition at 12 h after ischemic injury (upper panels). The corresponding analyses carried out in control kidneys are shown in the lower panels. (b) Immunohistochemical analyses of the renal outer medulla performed at a high-power view (original magnification, $\times 600$) by using antibodies against Notch-2, Delta-1, and DAPI and in a merged condition at 12 h after ischemic injury were carried out in AKI kidneys (upper panels) and in control kidneys (lower panels).

Figure 6. PMID: 18418349.

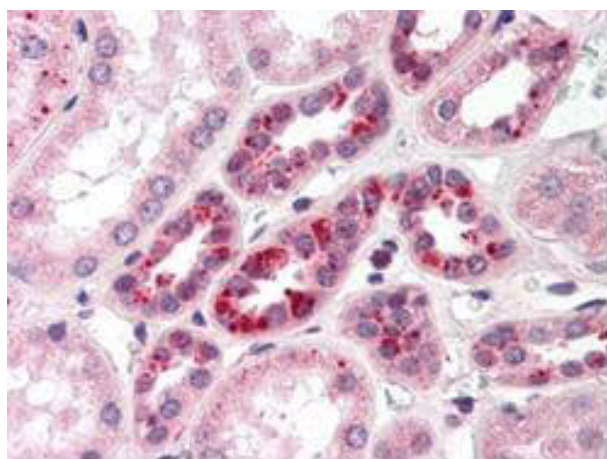


Immunofluorescence Microscopy

Expression of Notch receptors in endothelial and smooth muscle cells.

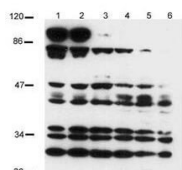
C. Immunofluorescence staining of Notch1, Notch2, and Notch3 in uninjured carotid artery (left panel) and ligated injured carotid artery (14 days after ligation, right panel) of WT mice. Vessels were co-stained for smooth muscle actin (SMA). SMA is stained red; Notch receptors were stained green; and nuclei were stained blue (DAPI). Yellow indicates presence of both red and green staining. White bar was 20 μm .

Fig 1. PMID: 19433762



Immunohistochemistry

Rockland's Anti-Notch 2 antibody was diluted 1:500 to detect NOTCH 2 in human kidney tissue. Tissue was formalin fixed and paraffin embedded. No pre-treatment of sample was required. The image shows the localization of antibody as the precipitated red signal, with a hematoxylin purple nuclear counter stain.



Western Blot

Western blot using Rockland's anti-Notch 2 (intra) antibody shows detection of a band at ~110 kDa corresponding to active Notch 2 protein. Western Blot analysis was performed for Notch 2 expression using 100µg of total protein lysate obtained from human mesothelial SV40 cells transfected with a plasmid encoding a constitutively active Notch 2 (intra cellular Notch 2). Lanes 1-3 contain lysate 24 h (1), 48 h (2), and 72 h (3) post transfection. Lanes 4-6 are the corresponding control cells (untransfected) taken at similar time points. The band at about 110kD represents active Notch 2. This band is not seen in the control cell. The intracellular domain of Notch 2 has a predicted band size of 110kD, corresponding to this band. Protein cell lysates were run on a 10% SDS-page gel, blotted onto Hybond C membrane, blocked overnight in PBS-Tween 20 supplemented with 5% Non-fat Milk and probed with anti-Notch 2 at a 1:400 dilution. ECL was used as visualization method.

References

- Gupta S et al. Effect of Notch activation on the regenerative response to acute renal failure. *Am J Physiol Renal Physiol* (2009)
- Li Y et al. Smooth Muscle Notch1 Mediates Neointimal Formation Following Vascular Injury. *Circulation*. (2009)
- Kobayashi T et al. Expression and function of the Delta-1/Notch-2/Hes-1 pathway during experimental acute kidney injury. *Kidney Int*. (2008)

Disclaimer

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