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# Datasheet for 100-401-407S NOTCH 1 Antibody

### **Overview**

Description:	Anti-NOTCH 1 (Cleaved N terminal) (Human specific) (RABBIT) Antibody - 100-401-407S
Item No.:	100-401-407S
Size:	25 μL
Applications:	Dot Blot, ELISA, IHC, WB, IF, IP, Multiplex
Reactivity:	Human, Mouse
Host Species:	Rabbit

## **Product Details**

Background:	Anti Notch 1 Antibody recognizes Notch 1 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.
Synonyms:	rabbit anti-notch1 antibody, Neurogenic locus Notch homolog protein 1, hN1, Translocation- associated Notch protein TAN-1
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

## **Target Details**

Gene Name:	NOTCH1
Reactivity:	Human, Mouse
Immunogen Type:	Conjugated Peptide



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Immunogen:	This whole rabbit serum was prepared by repeated immunizations with a synthetic peptide corresponding to amino acid residues of human Notch 1 located near the N-terminal sequence of the cleaved N intracellular domain (NICD).
Purity/Specificity:	This antiserum is directed against human NOTCH 1. Based on the immunogen sequence, we expect this antibody to react as well with mouse and rat NOTCH 1 (100% sequence homology). This antibody reacts with mouse Notch constructs present in lysates of HEK 293 cells. Only the cleaved intracellular (activated) form (NICD) is detected. No reactivity is detected against mouse N2, N3 or N4. The immunogen epitope is only exposed after gamma secretase cleavage and is not accessible in the uncleaved form.
Relevant Links:	<ul> <li>GeneID - 4851</li> <li>NCBI - NP_060087.3</li> <li>UniProtKB - P46531</li> </ul>

# **Application Details**

<b>Tested Applications:</b>	Dot Blot, ELISA, IHC, WB
Suggested Applications:	IF, IP, Multiplex (Based on references)
Application Note:	Anti-Notch 1 has been tested by ELISA, dot blot, western blot and immunohistochemistry. An 80 kDa band corresponding to Notch 1 was observed at a 1:500 dilution. 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:	1:20,000 - 1:60,000
IF:	User Optimized
IHC:	1:200
IP:	User Optimized
WB:	1:500- 1:2,000

## Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	90 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

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Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 $\mu$ L). To minimize loss of volume dilute 1:10 by adding 225 $\mu$ L of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

### **Shipping & Handling**

### Images



#### Western Blot

Systemic oestrogen signalling is mediated by EGFR and Notch. (A) Representative Western blot showing expression of cleaved (active) Notch1 (N1-ICD) following culture ± 1 nM  $17\beta$ -estradiol ± 10  $\mu$ M GSI. (Bi) Representative Western blot showing expression of Notch ligands in sorted MCF7 cells (left) and, where available, metastatic cells (right). (Bii) Densitometric analysis of three independent repeats of MCF7 sorting and of a single experiment for primary cells. Comparisons between population 1 (CSC enriched) and other populations are displayed. (C and D) Mammosphere formation was assessed following culture with 1 nM  $17\beta$ -estradiol ± gamma secretase inhibitor (GSI) alone and in combination with gefitinib. Fold change is normalised to control, untreated cells represented as line. (E) Representative image of protein levels of ERK and phosphorylated (actived) ERK following culture for 48 hours in monolayer  $\pm$  10  $\mu$ M GSI. Means plotted  $\pm$  SEM, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 compared to E2 treated. # P < 0.05 compared to control cells. Figure provided by CiteAb. Source: Breast Cancer Res, PMID: 23497505.

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# Western Blot

Rabbit anti-Human NOTCH 1 (Cleaved N Terminal) was used at a 1:500 dilution to detect mouse Notch 1 by Western blot. Equivalent amounts of lysates from transiently transfected 293 cells expressing recombinant myc-tagged mouse Notch constructs were electrophoresed and transferred to membrane using standard methods. A reaction with diluted primary antibody was followed by washing; reaction with a 1:10,000 dilution of HRP conjugated Gt-a-Rabbit IgG (611-103-122), and color development. 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. Personal communication, Dr. Stacey Huppert.

#### Immunohistochemistry

Immunohistochemistry of Rabbit anti-Notch1 antibody. Tissue: Exocrine glands of human pancreas. Fixation: FFPE. Primary antibody: Notch1 antibody at 1:200. Staining: moderate to strong membranous staining and faint to moderate cytoplasmic staining. Islets showed faint staining.



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### Western Blot

Dot Blot of Rabbit anti-Notch 1 (Cleaved N Terminal) (Human Specific) Antibody. Antigen: Row 1 - Notch 1 Peptide (Cleaved N Terminal) Row 2 - Notch 1 (Intra) Peptide. Load: Lane 1 - 200 ng Lane 2 - 66.67 ng Lane 3 - 22.22 ng Lane 4 -7.41 ng Lane 5 - 2.47 ng. Primary antibody: Rabbit anti-Notch 1 (Cleaved N Terminal) (Human Specific) Antibody at 1:1,000 for 60 min at RT. Secondary antibody: HRP Rabbit Secondary at 1:40,000 for 30 min at RT. Block: MB-070 for 1 HR at RT.



### References

#### Western Blot

Western Blot of Rabbit anti-Notch1 antibody. Lane 1: MCF-7 control lysate. Lane 2: MCF-7 +1 nM 17 $\beta$ -estradiol. Lane 3: MCF-7 + 10  $\mu$ M gamma secretase inhibitor. Load: 35  $\mu$ g per lane. Primary antibody: Notch1 antibody at 1:500 for overnight at 4°C. Secondary antibody: IRDye800<sup>TM</sup> rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 80 kDa for Notch1.



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