Order online now!

www.rockland.com
tech@rockland.com
+1 484.791.3823

Datasheet for 100-401-407

NOTCH 1 Antibody

Overview

Description:	Anti-NOTCH 1 (Cleaved N terminal) (Human specific) (RABBIT) Antibody - 100-401-407
Item No.:	100-401-407
Size:	200 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, NICD
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

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

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:	• UniProtKB - P46531 • NCBI - CAG33502.1 • GeneID - 4851

Application Details

Tested Applications:	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

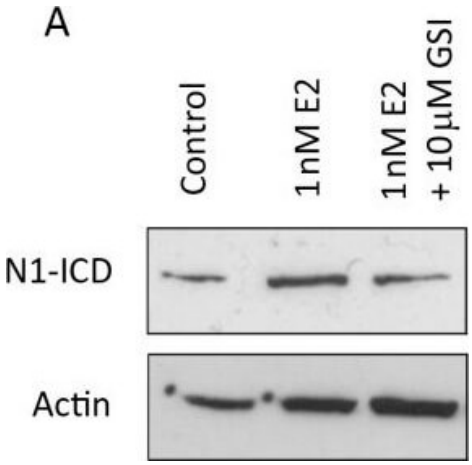
Formulation

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

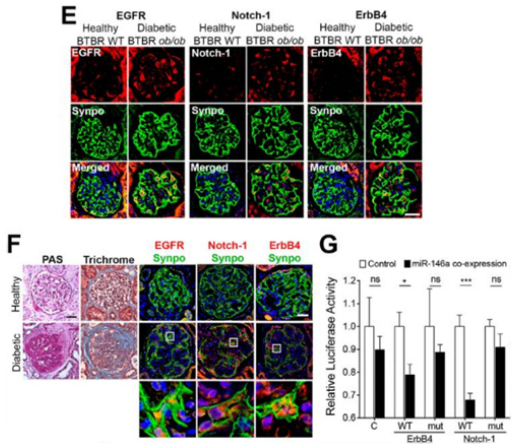
Shipping & Handling

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



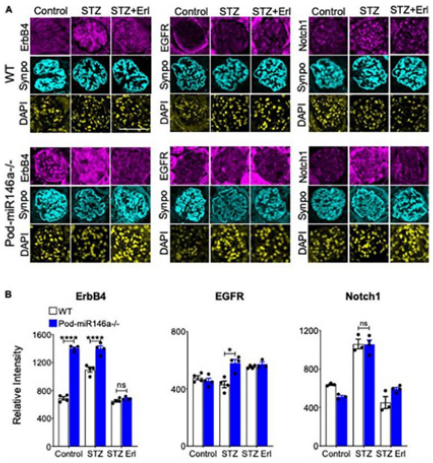
Western Blot
Systemic oestrogen signalling is mediated by EGFR and Notch. (A) Representative Western blot showing expression of cleaved (active) Notch1 (N1-ICD) following culture ± 1 nM 17β-estradiol ± 10 µM GSI. (Bi) Representative Western blot showing expression of Notch ligands in sorted MCF7 cells (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 17β-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 ± 10 µM GSI. Means plotted ± 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.



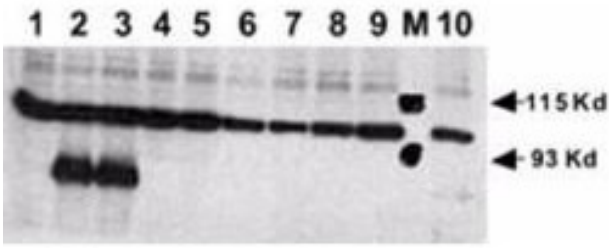
Immunofluorescence Microscopy

miR-146a targets Notch-1 and ErbB4 are up-regulated in the diseased glomeruli.

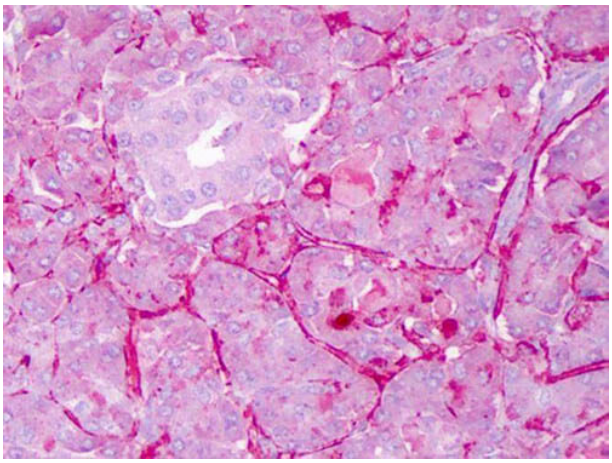
E). expression of EGFR, Notch-1, and ErbB4 is up-regulated in the glomeruli of BTBR ob/ob mice. Representative confocal microscopy images of immunofluorescently labeled glomeruli from 12-week-old BTBR WT (healthy) and BTBR ob/ob (diabetic) mice. Kidney sections were imaged after staining with DAPI and antibodies against EGFR, Notch-1, ErbB4, and Synaptopodin (Synpo). Merged DAPI, EGFR and Synpo, DAPI, Notch-1 and Synpo, and DAPI, ErbB4 and Synpo channels are also presented that show podocyte colocalization for these proteins. Scale bar, 50 µm. F). expression of EGFR, Notch-1, and ErbB4 is up-regulated in the glomeruli of diabetic patients. Representative images showing histochemical analyses of kidney tissue samples (left panels) after PAS and trichrome staining showing extensive glomerular expansion, mesangial sclerosis, and fibrosis in the diabetic kidneys. Scale bar, 50 µm. Representative confocal microscopy images of immunofluorescently labeled kidney sections that imaged after staining with DAPI and antibodies against EGFR, Notch-1, ErbB4 and Synpo (right panels). Merged EGFR and Synpo, Notch-1 and Synpo, and ErbB4 and Synpo channels are shown that show podocyte colocalization for these proteins, respectively. Scale bar, 50 µm. Bottom panels present higher magnification views of the boxed regions in the middle panel. G). miR-146a reduces the activity of luciferase linked with 3' UTRs of ErbB4 and Notch1. Bar graph reporting results from luciferase activity assay from HEK293T cells co-transfected with pre-miR-146a or pre-miR-control and a luciferase reporter plasmid containing either the WT or mutated (mut) sequence of 3' UTRs of ErbB4 or Notch-1. Luciferase activity was normalized with the activity of β-galactosidase from a co-transfected plasmid. Data shown are mean ± S.E. (n = 4). *, $p < 0.05$. ***, $p < 0.0005$. Fig 3. PMID: 27913625.



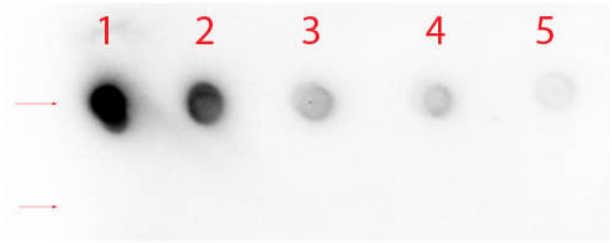
Immunofluorescence Microscopy
Immunofluorescence imaging-based analyses of glomerular sections shows erlotinib administration protects WT and Pod-miR-146a–/– mice from STZ injury via reduction in ErbB4 and EGFR. (A) Representative confocal microscopy images of immunofluorescently labeled glomeruli from WT (top three panels) and Pod-miR146a (bottom three panels) mice treated with vehicle alone (Control), with STZ and vehicle (STZ) or with STZ and erlotinib (STZ Erl). Tissue sections were imaged after staining with DAPI (nuclear marker) and antibodies against ErbB4, EGFR, Notch-1 and Synaptopodin (Synpo, podocyte marker) (as indicated). Scale bar, 50 µm. (B) Bar graphs showing quantification of relative glomerular signal intensity of ErbB4, EGFR and Notch-1 in tissue samples from A. Statistics were performed using two-way ANOVA. Data shown are mean ± SEM (n = 5/group). *p < 0.05; ***p < 0.001; ns, no significant difference. Fig 5. PMID: 36059820.



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.



Dot 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

- Li X et al. Podocyte-specific deletion of miR-146a increases podocyte injury and diabetic kidney disease. *Front Med (Lausanne).* (2022)
- Iervolino A et al. Potassium depletion induces cellular conversion in the outer medullary collecting duct altering Notch signaling pathway. *Sci Rep.* (2020)
- Lee et al. Absence of miR-146a in Podocytes Increases Risk of Diabetic Glomerulopathy via Up-regulation of ErbB4 and Notch-1. *Journal of Biological Chemistry* (2017)
- Manderfield et al. Hippo signaling is required for Notch-dependent smooth muscle differentiation of neural crest. *Development* (2015)
- Harrison H et al. Contrasting hypoxic effects on breast cancer stem cell hierarchy is dependent on ER-α status. *Cancer Res* (2013)
- Harrison H et al. Oestrogen increases the activity of oestrogen receptor negative breast cancer stem cells through paracrine EGFR and Notch signalling. *Breast Cancer Res* (2013)
- Poli EF et al. Murine neural stem cells model Hunter disease in vitro: glial cell-mediated neurodegeneration as a possible mechanism involved. *Cell Death Dis* (2013)
- Gupta S et al. Effect of Notch activation on the regenerative response to acute renal failure. *Am J Physiol Renal Physiol* (2009)
- Solomon A et al. Upregulation of the let-7 microRNA with precocious development in lin-12/Notch hypermorphic Caenorhabditis elegans mutants. *Dev Biol.* (2008)

Disclaimer