

# Anti-Notch 1 Antibody [SJ205] - BSA and Azide free

## HA750111



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
<b>Molecular Wt:</b>	Predicted band size: 273 kDa
<b>Clone number:</b>	SJ205

**Description:** The LIN-12/notch family of transmembrane receptors is believed to play a central role in development by regulating cell fate decisions. To date, four notch homologs have been identified in mammals and have been designated Notch 1, Notch 2, Notch 3 and Notch 4. The notch genes are expressed in a variety of tissues in both the embryonic and adult organism, suggesting that the genes are involved in multiple signaling pathways. The notch proteins have been found to be overexpressed or rearranged in human tumors. Ligands for notch include Jagged1, Jagged2 and Delta. Jagged can activate notch and prevent myoblast differentiation by inhibiting the expression of muscle regulatory and structural genes. Jagged2 is thought to be involved in the development of various tissues whose development is dependent upon epithelial-mesenchymal interactions. Normal Delta expression is restricted to the adrenal gland and placenta. Delta expression has also been found in neuroendocrine tumors such as neuroblastomas and pheochromocytomas.

**Immunogen:** Synthetic peptide within Human Notch 1 aa 2,481-2,530 / 2,555.

**Positive control:** C6 cell lysate, rat brain tissue lysate, HeLa cell lysate, THP-1 cell lysate, A549 cell lysate, HEK-293 cell lysate, Hela, HepG2, NIH/3T3, human pancreas tissue, mouse lung tissue, mouse pancreas tissue.

**Subcellular location:** Cell membrane, Late endosome membrane; Nucleus (intracellular domain).

**Database links:** SwissProt: P46531 Human | Q01705 Mouse | Q07008 Rat

### Recommended Dilutions:

<b>WB</b>	1:1,000-1:5,000
<b>IF-Cell</b>	1:50-1:200
<b>IF-Tissue</b>	1:50-1:200
<b>IHC-P</b>	1:50-1:200
<b>FC</b>	1:50-1:100
<b>IP</b>	Use at an assay dependent concentration.

**Storage Buffer:** 1\*PBS (pH7.4).

**Storage Instruction:** Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

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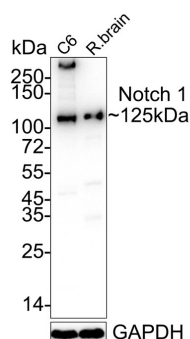
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## Images



**Fig1:** Western blot analysis of Notch 1 on different lysates with Rabbit anti-Notch 1 antibody (HA750111) at 1/5,000 dilution.

Lane 1: C6 cell lysate (20  $\mu$ g/Lane)

Lane 2: Rat brain tissue lysate (40  $\mu$ g/Lane)

Predicted band size: 273 kDa

Observed band size: 125/273 kDa

Exposure time: 1 minute; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750111) at 1/5,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

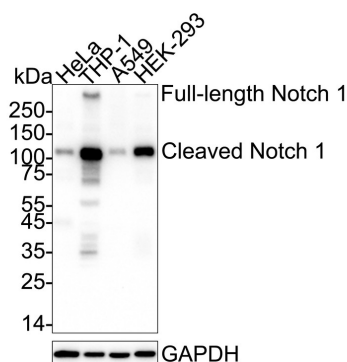
**Fig2:** Western blot analysis of Notch 1 on different lysates with Rabbit anti-Notch 1 antibody (HA750111) at 1/1,000 dilution.

Lane 1: HeLa cell lysate

Lane 2: THP-1 cell lysate

Lane 3: A549 cell lysate

Lane 4: HEK-293 cell lysate



Lysates/proteins at 30  $\mu$ g/Lane.

Predicted band size: 273 kDa

Observed band size: 125/273 kDa

Exposure time: 59 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750111) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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**Fig3:** Western blot analysis of Notch 1 on different lysates with Rabbit anti-Notch 1 antibody (HA750111) at 1/1,000 dilution.

Lane 1: HeLa-si NT cell lysate

Lane 2: HeLa-si Notch 1 cell lysate

Lysates/proteins at 20 µg/Lane.

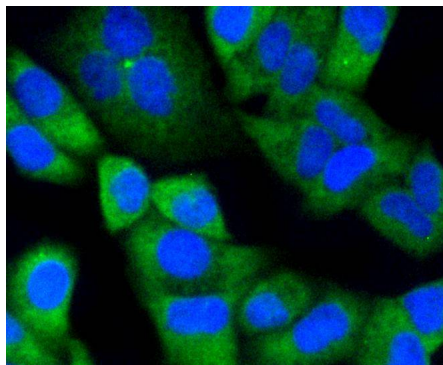
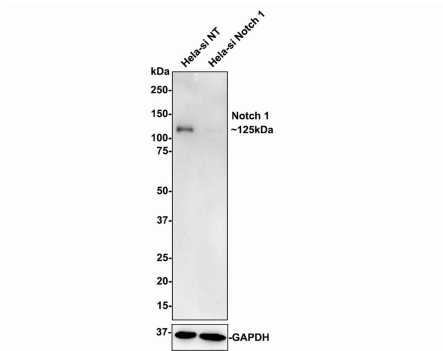
Predicted band size: 273 kDa

Observed band size: 125 kDa

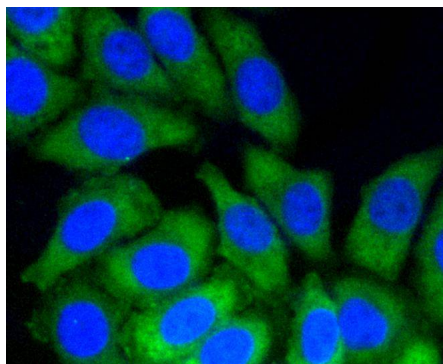
Exposure time: 6 minutes;

4-20% SDS-PAGE gel.

ET1606-55 was shown to specifically react with Notch 1 in HeLa-si NT cells. Weakened band was observed when HeLa-si Notch 1 sample was tested. HeLa-si NT and HeLa-si Notch 1 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFD in TBST for 1 hour at room temperature. The primary antibody (ET1606-55, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.



**Fig4:** ICC staining of Notch 1 in HeLa cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750111, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig5:** ICC staining of Notch 1 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750111, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

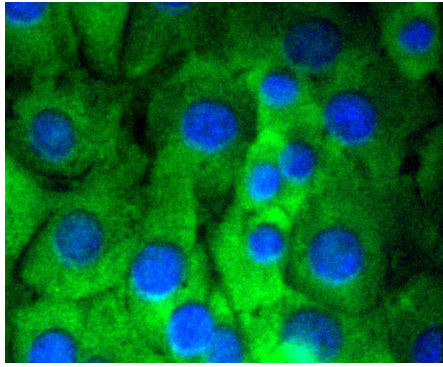
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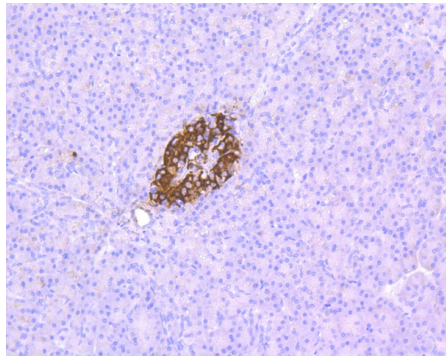
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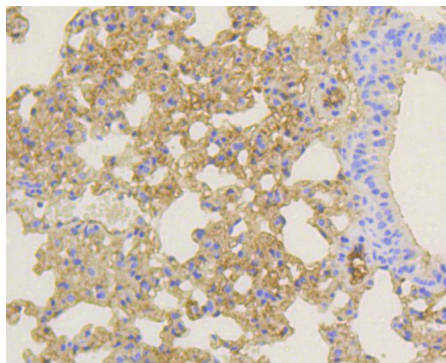
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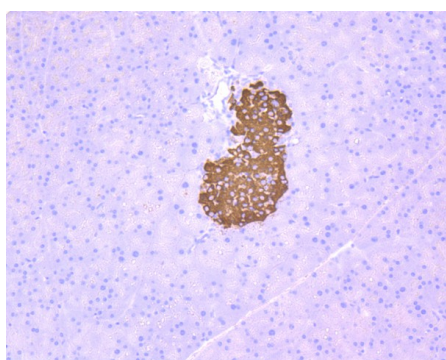
**Fig6:** ICC staining of Notch 1 in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750111, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



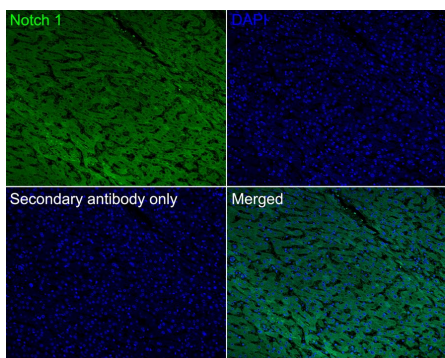
**Fig7:** Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-Notch 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750111, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig8:** Immunohistochemical analysis of paraffin-embedded mouse lung tissue using anti-Notch 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750111, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

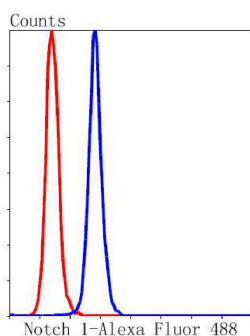


**Fig9:** Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue using anti-Notch 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750111, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig10:** Immunofluorescence analysis of paraffin-embedded human liver cancer tissue labeling Notch 1 with Rabbit anti-Notch 1 antibody (HA750111) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA750111, green) at 1/50 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).



**Fig11:** Flow cytometric analysis of Notch 1 was done on HeLa cells. The cells were fixed, permeabilized and stained with the primary antibody (HA750111, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes. Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

**Note:** All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

## Background References

1. Li W et al. Genome-wide analyses identify KLF4 as an important negative regulator in T-cell acute lymphoblastic leukemia through directly inhibiting T-cell associated genes. *Mol Cancer* 14:26 (2015).
2. Nakajima K et al. Galectin-3 inhibits osteoblast differentiation through notch signaling. *Neoplasia* 16:939-49 (2014).

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