

Product Information

Description:	This anti-Notch (neurogenic locus notch homolog protein) antibody is a purified, recombinant monoclonal antibody that recognizes human Notch1, Notch2 and Notch3. This recombinant antibody has been tested for specific binding to purified recombinant Notch1/2/3 by ELISA. The antibody was affinity purified.
Background:	The Notch (neurogenic locus notch homolog protein) signaling pathway controls cell fate decisions in vertebrates and invertebrates and is involved in embryonic development, tissue homeostasis, and regulation of the immune and angiogenic systems. Notch signaling is triggered through the binding of a transmembrane ligand, present in opposing cells, to one of the four existing Notch transmembrane receptors (Notch1/Notch2/Notch3/Notch4). This results in proteolytic cleavage of the Notch receptor, releasing the constitutively active intracellular domain of the Notch receptor (NICD). NICD translocate to the nucleus and associates with the transcription factor CSL (CBF1/RBPJk/Suppressor of Hairless/Lag-1) and coactivator Mastermind to turn on the transcription of Notch-responsive genes. Dysfunction of Notch signaling has severe consequences, including developmental pathologies or cancer (such as T cell acute lymphoblastic leukemia, T-ALL, and urothelial bladder cancer). The use of Notch inhibitors, mainly gamma secretase inhibitors, as a cancer therapy option and in the regeneration of tissues is under investigation. Further studies will allow us to have a deeper understanding of Notch signaling and will benefit future therapeutic approaches.
Species:	Human
Isotype:	IgG1
Clonality:	Monoclonal
Concentration:	0.50 mg/ml
Expression System:	HEK293
Purity:	≥90%
Format:	Aqueous buffer solution.
Formulated In:	8 mM phosphate, pH 7.4, 110 mM NaCl, 2.2 mM KCl, and 20% glycerol
MW:	Heavy Chain: 48 kDa + glycans; Light Chain: 23 kDa
Glycosylation:	This antibody has a higher MW by SDS-PAGE due to glycosylation.
Stability:	At least 12 months at -80°C.
Storage:	-80°C
Instructions for Use:	Thaw on ice and gently mix prior to use. DO NOT VORTEX. Perform a quick spin before opening. Aliquot into small volumes and flash freeze for long term storage. Avoid multiple freeze/thaw cycles.
Assay Conditions:	Notch1 and Notch3 Binding Validation: The antibody was validated by measuring Anti-Notch Neutralizing Antibody binding to Notch1 and Notch3 by ELISA. Notch1 (#101897) and Notch3 (#101992) were coated onto a 96-well plate overnight at 4°C (50 µl/well at a concentration of 2 µg/ml in PBS). The plate was washed 3 times with Immuno Buffer 1 (#79311) and blocked using 100 µl of Blocking Buffer 2 (#79728) for 1 hour at Room Temperature (RT). After removing the blocking buffer, 50 µl/well of purified Anti-Notch Neutralizing Antibody (#102065), serially diluted in Blocking Buffer 2, was added for 1 hour at RT. After 3 more washes, the plate was incubated with anti-human IgG Fc-HRP, washed, and incubated with the Colorimetric HRP substrate. The reaction was stopped, and absorbance was read at 450 nm. The Blank value was subtracted from all values.

Notch2 Binding Validation: The antibody was validated by measuring Anti-Notch Neutralizing Antibody (#102065) binding to Notch2 by ELISA. Anti-Notch Neutralizing Antibody (#102065) was coated onto a 96-well plate overnight at 4°C (50 µl/well at a concentration of 2 µg/ml in PBS). The plate was washed 3 times with Immuno Buffer 1 (#79311) and blocked using 100 µl of Blocking Buffer 2 (#79728) for 1 hour at Room Temperature (RT). After removing the blocking buffer, 50 µl/well of purified Notch2 (#101987), serially diluted in Blocking Buffer 2, was added for 1 hour at RT. After 3 more washes, the plate was incubated with Streptavidin-HRP (#79742), washed, and incubated with the Colorimetric HRP substrate. The reaction was stopped, and absorbance was read at 450 nm. The Blank value was subtracted from all values.

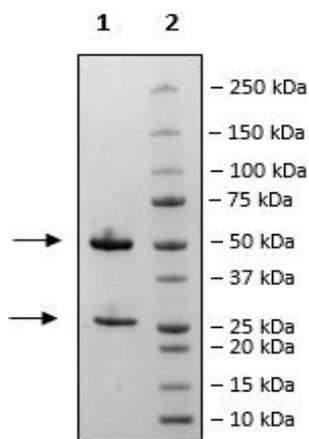
Notch1/DLL4 Neutralization Assay: This assay was performed following the protocol described in Notch1:DLL4 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit (#82284) with increasing concentrations of Anti-Notch Neutralizing Antibody.

Applications:

Useful for binding studies and as positive control in neutralization assays.

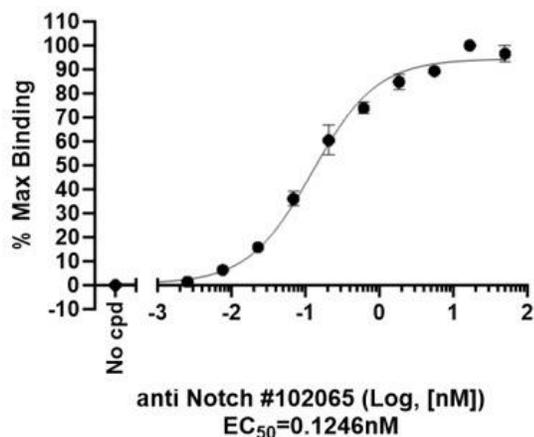
Quality Control Data

4-20% SDS-PAGE Coomassie Staining

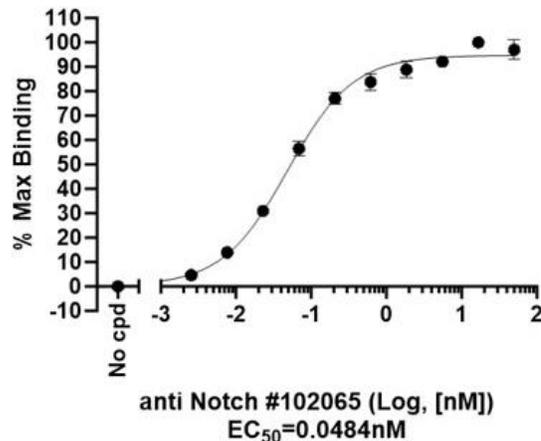


Quality Control Data

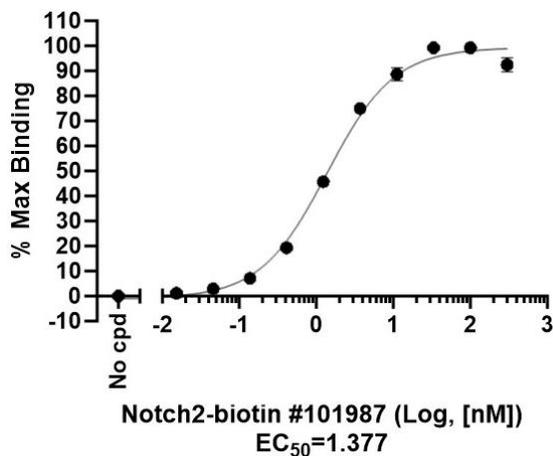
Notch1:Anti Notch Binding Assay



Notch3:Anti Notch Binding Assay



Anti Notch:Notch2-Biotin Binding Assay



Notch1:DLL4-Biotin Neutralization Assay

