

DESCRIPTION

Species Reactivity	Mouse/Rat
Specificity	Detects rat Notch-1 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 5% cross-reactivity with recombinant rat Notch-2 and recombinant mouse Notch-3 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant rat Notch-1 Arg20-Glu488 (Ala208Thr, Asp334Glu) Accession # Q07008
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

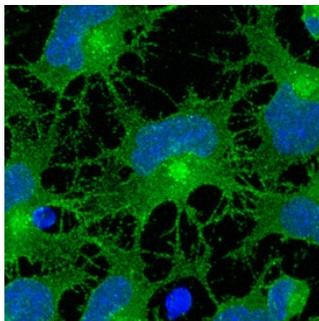
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	Recombinant Rat Notch-1 Fc Chimera (Catalog # 1057-TK)
Flow Cytometry	2.5 µg/10 ⁶ cells	Rat cortical stem cells
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Blockade of Receptor-ligand Interaction	In a functional ELISA, 1-3 µg/mL of Goat Anti-Mouse/Rat Notch-1 Antibody (Catalog # AF1057) will block 50% of the binding of 200 ng/mL of Recombinant Rat Jagged 1 Fc Chimera (Catalog # 599-JG) to immobilized Recombinant Rat Notch-1 Fc Chimera (Catalog # Catalog # 1057-TK) coated at 5 µg/mL (100 µL/well). At 20 µg/mL, this antibody will block >80% of the binding.	

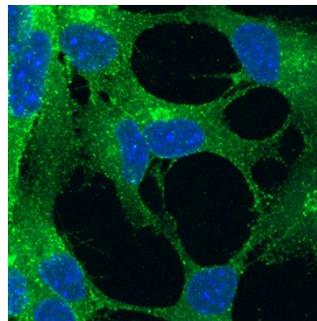
DATA

Immunocytochemistry



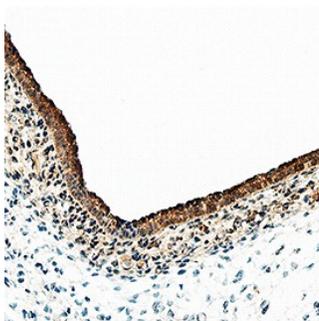
Notch-1 in Rat Cortical Stem Cells. Notch-1 was detected in immersion fixed undifferentiated rat cortical stem cells using Goat Anti-Mouse/Rat Notch-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1057) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 493-conjugated Anti-Goat IgG Secondary Antibody (green; Catalog # NL003) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for [Fluorescent ICC Staining of Stem Cells on Coverslips](#).

Immunocytochemistry



Notch-1 in Mouse Cortical Stem Cells. Notch-1 was detected in immersion fixed undifferentiated mouse cortical stem cells using Goat Anti-Mouse/Rat Notch-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1057) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 493-conjugated Anti-Goat IgG Secondary Antibody (green; Catalog # NL003) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for [Fluorescent ICC Staining of Stem Cells on Coverslips](#).

Immunohistochemistry



Notch-1 in Rat Embryo. Notch-1 was detected in immersion fixed paraffin-embedded sections of rat embryo (13 d.p.c.) using Goat Anti-Mouse/Rat Notch-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1057) at 10 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to labeling in epidermis. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Rat Notch-1 is a 300 kDa, type I transmembrane glycoprotein involved in a number of early-event developmental processes (1). In both vertebrates and invertebrates, Notch signaling is important for specifying cell fates and for defining boundaries between different cell types. The molecule is synthesized as a 2531 amino acid (aa) precursor that contains an 18 aa signal sequence, a 1705 aa extracellular region, a 23 aa transmembrane (TM) segment and a 785 aa cytoplasmic domain (2). The large Notch-1 extracellular domain has 36 EGF-like repeats followed by three notch/Lin-12 repeats. Of the 36 EGF-like repeats, the 11th and 12th EGF-like repeats have been shown to be both necessary and sufficient for binding the ligands Delta and Serrate, in *Drosophila* (3). The Notch-1 cytoplasmic domain contains six ankyrin repeats, a glutamine-rich domain and a PEST sequence. The Notch-1 receptor undergoes post-translational proteolytic cleavage by a furin-like enzyme to form a heterodimer of the 1635 aa ligand binding extracellular region and the 877 aa transmembrane protein (4). Upon ligand binding, additional sequential proteolysis by TNF-converting enzyme and the Presenilin-dependent γ -secretase results in the release of the Notch intracellular domain (NICD) which translocates into the nucleus where it functions as a transcription activator to initiate transcription of Notch-responsive genes (5). An alternative Notch signaling pathway that is mediated by the full-length form of Notch that has not been cleaved by the furin-like enzyme has also been reported (6). The rat Notch-1 extracellular domain shows 86% and 97% aa identity to human and mouse Notch-1 extracellular domains respectively. It also exhibits 56% and 50% aa identity with rat Notch-2 and Notch-3 extracellular domains, respectively.

References:

1. Weinmaster, G. (2000) *Curr. Opin. Genet. Dev.* **10**:363.
2. Weinmaster, G. *et al.* (1991) *Development* **113**:199.
3. Rebay, I. *et al.* (1991) *Cell* **67**:687.
4. Rogeat, F. *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**:8108.
5. Mumm, J.S. and R. Kopan (2000) *Dev. Biol.* **228**:151.
6. Bush, G. *et al.* (2001) *Dev. Biol.* **229**:494.