Rat Notch-1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1057

DESCRIPTION

Species Reactivity	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.

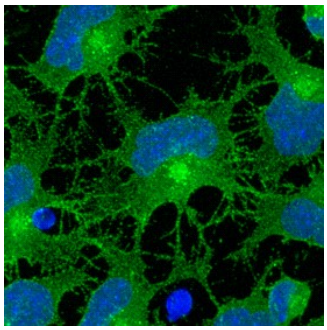
APPLICATIONS

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)
Blockade of Receptor-ligand Interaction	1 - 3 µg/mL	At 20 µg/mL, this antibody will block > 80% of the binding.
Flow Cytometry	2.5 µg/10^6 cells	Rat cortical stem cells
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	Immersion fixed frozen sections of embryonic rat brain (E19)

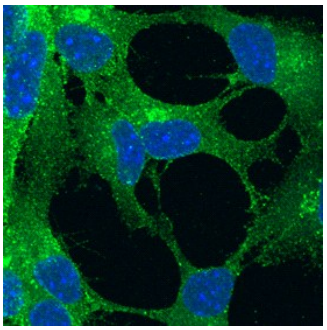
DATA

Immunocytochemistry



Notch-1 in Rat Cortical Stem Cells. Notch-1 was detected in immersion fixed undifferentiated rat cortical stem cells using Goat Anti-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-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.

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.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 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.

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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 (NCID) 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.