

DESCRIPTION

Species Reactivity	Human
Specificity	Detects the intracellular domain (ICD) of human Notch-1 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 1% cross-reactivity with recombinant human (rh) Notch-2 ICD, rhNotch-3 ICD, and rhNotch-4 ICD is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Notch-1 Gly2428-Lys2556 Accession # P46531
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 as a 0.2 µm filtered solution in PBS.

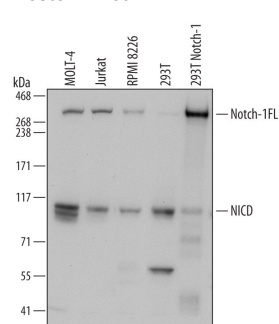
APPLICATIONS

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	1 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	5 µg/5 x 10 ⁶ cells	See Below
Flow Cytometry	2.5 µg/10 ⁶ cells	U2OS human osteosarcoma cell line
Immunocytochemistry	5-15 µg/mL	See Below

DATA

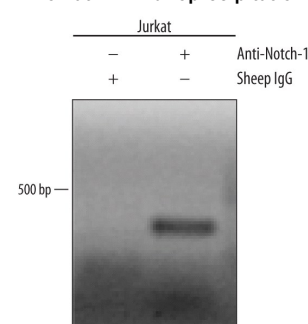
Western Blot



Detection of Human Notch-1.

Western blot shows lysates of MOLT-4 human acute lymphoblastic leukemia cell line, Jurkat human acute T cell leukemia cell line, RPMI 8226 human multiple myeloma cell line, 293T human embryonic kidney cell line, and 293T human embryonic kidney cell line (1 µg per lane), transfected with full length human Notch-1. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human Notch-1 Intracellular Domain Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3647) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for Notch-1 intracellular domain (NICD) and full length Notch-1 (Notch-1 FL) at approximately 115 and 300 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

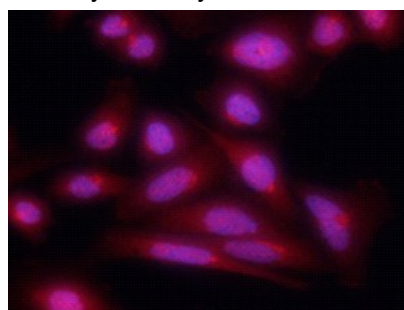
Chromatin Immunoprecipitation (ChIP)



Detection of Notch-1-regulated Genes by Chromatin Immunoprecipitation.

Jurkat human acute T cell leukemia cell line treated with 50 ng/mL PMA and 200 ng/mL calcium ionomycin for 30 minutes was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. Notch-1/DNA complexes were immunoprecipitated using 5 µg Sheep Anti-Human Notch-1 Intracellular Domain Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3647) or control antibody (Catalog # 5-001-A) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Sheep IgG Secondary Antibody (Catalog # BAF016). Immunocomplexes were captured using 50 µL of MagCollect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *c-myc* promoter was detected by standard PCR.

Immunocytochemistry



Notch-1 in Saos-2 Human Cell Line.

Notch-1 was detected in immersion fixed Saos-2 human osteosarcoma cell line using 10 µg/mL Sheep Anti-Human Notch-1 Intracellular Domain Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3647) for 3 hours at room temperature. Cells were stained with the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of 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. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <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

Notch-1 (so named for "notches" in fly wings; also TAN-1) is a 300 kDa member of the Notch family of glycoproteins. It is associated with gene activation in both embryo and adult. Human Notch-1 is a 2538 amino acid (aa) type I transmembrane glycoprotein. It undergoes Golgi processing to generate a heterodimer composed of a 180-200 kDa disulfide-linked extracellular domain (aa 18-1664) and a 120 kDa membrane-bound segment (aa 1665-2556). Upon ligand binding, the 110 kDa segment undergoes two cleavages which generate an NICD (notch intracellular domain) (aa 1754-2556), a nuclear transcription factor. One isoform shows a deletion of aa 248-288. Over aa 2428-2556, human Notch 1 is 83% and 89% aa identical to canine and mouse Notch-1, respectively.