#3640 Store at -20°C

Notch Isoform Antibody Sampler Kit

☑ 1 Kit
(4 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Cleaved Notch1 (Val1744) (D3B8) Rabbit mAb	4147	20 µl	110 kDa	Rabbit IgG
Notch1 (D1E11) XP® Rabbit mAb	3608	20 µl	120, 300 kDa	Rabbit IgG
Notch2 (D76A6) XP® Rabbit mAb	5732	20 µl	110, 300 kDa	Rabbit IgG
Notch3 (D11B8) Rabbit mAb	5276	20 µl	90, 270 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Notch Isoform Antibody Sampler Kit provides an economical means to investigate Notch Signaling. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Notch proteins (Notch1-4) are a family of transmembrane receptors that play important roles in development and the determination of cell fate (1). Mature Notch receptors are processed and assembled as heterodimeric proteins, with each dimer comprised of a large extracellular ligand-binding domain, a single-pass transmembrane domain, and a smaller cytoplasmic subunit (Notch intracellular domain, NICD) (2). Binding of Notch receptors to ligands of the Delta-Serrate-Lag2 (DSL) family triggers heterodimer dissociation, exposing the receptors to proteolytic cleavages; these result in release of the NICD, which translocates to the nucleus and activates transcription of downstream target genes (3-4).

Constitutively activated Notch1 signaling is associated with the majority of cases of T cell acute lymphoblastic leukemia (T-ALL). The activation is either due to mutations in Notch1 itself or in the components of ubiquitin ligase complex, namely FBW7 (5-6). Notch2 is a member of the Notch family and mutation in Notch2 is associated with Alagille syndrome (7). Notch3 is a member of the Notch family and is processed similar to Notch1 (8). It is expressed primarily in arterial smooth muscle cells (SMC). Mutations altering the number of cysteine residues in the notch3 extracellular region are associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a hereditary angiopathy leading to strokes and dementia in adults (9-11). Recent studies indicate that Notch3 is overexpressed in many types of cancer (12-14).

Specificity/Sensitivity: Cleaved Notch1 (V1744) (D3B8) Rabbit mAb detects endogenous levels of the Notch1 intra- cellular domain (NICD) only when released by cleavage between Gly1753 and Val1754 (equivalent to Gly1743/Val1744 of murine notch1). The antibody does not recognize full- length Notch1 or Notch1 cleaved at other positions. The size of the NICD varies among cell lines due to mutations in the Notch1 C-terminus (6). Notch1 (D1E11) XP® Rabbit mAb detects endogenous levels of total Notch1 protein. It recognizes both the full-length (~300 kDa) and the trans- membrane/intracellular region NTM (~120 kDa). Notch2 (D76A6) XP® Rabbit mAb detects endogenous levels of total Notch2 protein. It recognizes both the full-length (~ 300 kDa) and the trans- membrane/intracellular region NTM (~110 kDa). Notch3 (D11B8) Rabbit mAb detects endogenous levels of total Notch3 protein. The antibody recognizes both full-length (FL) Notch3 at 270 kDa and a truncated protein (NTM) containing a short extracellullar region, the transmembrane domain and the intracellular region at 90 kDa.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the sequence at the Val1754 cleavage site in human Notch1 (equivalent to Val1744 in mouse Notch1), Pro2438 of human Notch1, Ala2738 of human Notch2, or Glu2312 of human Notch3 protein.

Background References:

(1) Artavanis-Tsakonas, S. et al. (1999) *Science* 284, 770-6.
(2) Chan, Y.M. and Jan, Y.N. (1998) *Cell* 94, 423-6.
(3) Schroeter, E.H. et al. (1998) *Nature* 393, 382-6.
(4) Rand, M.D. et al. (2000) *Mol Cell Biol* 20, 1825-35.
(5) Weng, A.P. et al. (2004) *Science* 306, 269-71.
(6) Thompson, B.J. et al. (2007) *J Exp Med* 204, 1825-35.
(7) McDaniell, R. et al. (2006) *Am J Hum Genet* 79, 169-73.
(8) Baron, M. (2003) *Semin Cell Dev Biol* 14, 113-9.
(9) Kalimo, H. et al. (2002) *Brain Pathol* 12, 371-84.
(10) Karlström, H. et al. (2002) *Proc Natl Acad Sci USA* 99, 17119-24.
(11) Monet, M. et al. (2007) *Hum Mol Genet* 16, 982-92.
(12) Park, J.T. et al. (2006) *Cancer Res* 66, 6312-8.
(13) Gramantieri, L. et al. (2007) *Liver Int* 27, 997-1007.
(14) Yamaguchi, N. et al. (2008) *Cancer Res* 68, 1881-8.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.