Store at -20°C

#68309

Notch Activated Targets Antibody Sampler Kit

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
c-Myc (D84C12) Rabbit mAb	5605	20 µl	57–65 kDa	Rabbit IgG
Cyclin D3 (DCS22) Mouse mAb	2936	20 µl	31 kDa	Mouse IgG1
HES1 (D6P2U) Rabbit mAb	11988	20 µl	30 kDa	Rabbit IgG
MAML1 (D3K7B) Rabbit mAb	12166	20 µl	130 kDa	Rabbit IgG
Notch1 (D1E11) XP® Rabbit mAb	3608	20 µl	100, 300 kDa	Rabbit IgG
Cleaved Notch1 (Val1744) (D3B8) Rabbit mAb	4147	20 µl	110 kDa	Rabbit IgG
p21 Waf1/Cip1 (12D1) Rabbit mAb	2947	20 µl	21 kDa	Rabbit IgG
RBPSUH (D10A4) XP® Rabbit mAb	5313	20 µl	61 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

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, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

Recommended Antibody Dilutions:

Western blotting	1:1000

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Description: The Notch Activated Targets Antibody Sampler Kit provides an economical means of detecting target proteins of activated Notch. The kit contains enough primary antibody to perform two western blot experiments per 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). RBPSUH (Recombining Binding Protein, SUppressor of Hairless), is the DNA-binding component of the transcription complex regulated by canonical Notch signaling. Binding of Notch with RBPSUH activates a transcription activation complex that includes Mastermind-like (MAML) proteins, leading to transcriptional activation of Notch target genes (5-7). The NICD binds and activates c-Myc which functions as a transcriptional regulator with roles in various aspects of cell behavior including proliferation, differentiation and apoptosis (8). The tumor suppressor protein p21 Waf1/Cip1 acts as an inhibitor of cell cycle progression. The NICD-RBPSUH complex binds and activates p21 for transcription (15). HES1 (Hairy and Enhancer of Split 1) is one of seven members of the HES family of basic helix-loop-helix (bHLH) transcription factors that is particularly well known as a repressive mediator of the canonical Notch signaling pathway (10). HES1 plays a key role in mediating Notch-dependent T cell lineage commitment (11), and has been reported to be an essential mediator of Notch-induced T cell acute lymphoblastic leukemia (T-ALL) (11,12). The active complex of cyclin D/CDK4 targets the retinoblastoma protein for phosphorylation, allowing the release of E2F transcription factors that activate G1/S-phase gene expression (13). Transcription of cyclin D is in part regulated by the NICD binding to the promoter region of cyclin D (14).

Specificity/Sensitivity: Notch1 (D1E11) XP® Rabbit mAb detects intracellular epitopes between 2400 and 2500 amino acids of human Notch1. It recognizes both the full-length (~300 kDa) and the NTM region (~120 kDa). The antibody cannot detect the extracellular (ligand-binding) domain of Notch1 following cleavage at the S2 site by ADAM-type metalloproteases. Cleaved Notch1 (V1744) (D3B8) Rabbit mAb detects endogenous levels of the Notch1 intracellular 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. RBPSUH (D10A4) XP® Rabbit mAb recognizes endogenous levels of total RBPSUH protein. MAML1 (D3K7B) Rabbit mAb recognizes endogenous levels of total MAML1 protein. This antibody does not detect MAML2 or MAML3. c-Myc (D84C12) Rabbit mAb detects endogenous levels of total c-Myc protein. p21 Waf1/Cip1 (12D1) Rabbit mAb detects endogenous levels of total p21 protein. The antibody does not cross-react with other CDK inhibitors. HES1 (D6P2U) Rabbit mAb recognizes endogenous levels of total HES1 protein. Cyclin D3 (DCS22) Mouse mAb detects endogenous levels of total cyclin D3 protein. The antibody does not cross-react with cyclin D1 or cyclin D2.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro2438 of human Notch1, the Val1754 cleavage site in human Notch1 (equivalent to Val1744 in mouse Notch1), residues surrounding Gln110 of human RBPSUH protein, residues surrounding Asp269 of human MAML1 protein, amino-terminal residues of c-Myc, residues near the carboxy-terminus of human p21, recombinant protein specific to human HES1 protein, residues 241-260 of recombinant human cyclin D3. Antibodies are purified by protein A and peptide affinity chromatography.

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) Wu, L. et al. (2002) *Mol Cell Biol* 22, 7688-700.
(6) Lin, S.E. et al. (2002) *J Biol Chem* 277, 50612-20.
(7) Kitagawa, M. et al. (2001) *Mol Cell Biol* 21, 4337-46.
(8) Baudino, T.A. and Cleveland, J.L. (2001) *Mol Cell Biol* 21, 691-702.
(9) Flores-Rozas, H. et al. (1994) *Proc Natl Acad Sci USA* 91, 8655-9.
(10) Kobayashi, T. and Kageyama, R. (2010) *Genes Cells* 15, 689-98.
(11) Wendorff, A.A. et al. (2010) *Immunity* 33, 671-84.
(12) Espinosa, L. et al. (2010) *Cancer Cell* 18, 268-81.
(13) Lukas, J. et al. (1996) *Mol Cell Biol* 16, 6917-25.
(14) Li, X. and von Boehmer, H. (2011) *ISRN Hematol* 2011, 921706.
(15) Niimi, H. et al. (2007) *J Cell Biol* 176, 695-707.

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

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:** (#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.

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