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Cleaved Notch1 (Val1744) (D3B8) Rabbit mAb



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rev. 01/19/16

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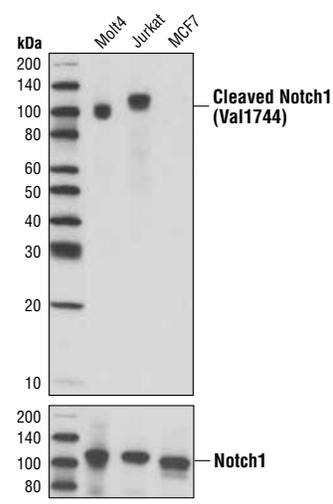
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, ChIP Endogenous	H, M, R	110 kDa	Rabbit IgG**

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).

The NICD of murine Notch1 is released (activated) by cleavage between Gly1743 and Val1744 (corresponding to Gly1753/Val1754 in human Notch1) (3, 4). Mutations that result in constitutive activation of Notch1 are associated with many different cancers, including a majority of cases of T cell acute lymphoblastic leukemia (T-ALL). Activation may be due to mutations in Notch1 itself, or in components of the ubiquitin ligase complex that negatively regulates the Notch signaling pathway (5-6).

Specificity/Sensitivity: 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 (6).

Source/Purification: Monoclonal antibody is 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).



Western blot analysis of extracts from various cell lines using Cleaved Notch1 (V1744) (D3B8) Rabbit mAb (upper) or Notch1 (D1E11) XP™ Rabbit mAb #3608 (lower).

Entrez-Gene ID #4851
 Swiss-Prot Acc. #P46531

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.

***Species cross-reactivity is determined by western blot.**
****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

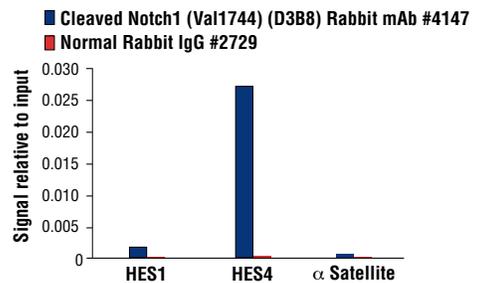
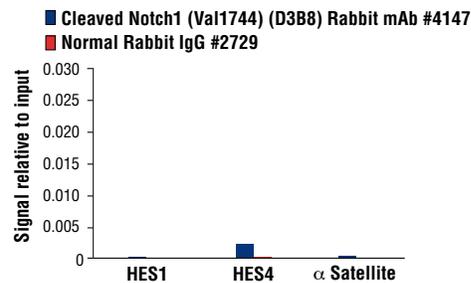
Western blotting	1:1000
Immunoprecipitation	1:200
Chromatin IP	1:200

For application specific protocols please see the web page for this product at www.cellsignal.com.

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

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.



CUTLL1 cells were cultured in media with γ -secretase inhibitor (1 μ M) for 3 days and then either harvested immediately (left panel) or washed and cultured in fresh media for 3 h (right panel). Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 cells and 2.5 μ l of Cleaved Notch1 (Val1744) (D3B8) Rabbit mAb or 2 μ l of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using human HES1 promoter primers, SimpleChIP® Human HES4 Promoter Primers #7273, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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—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.

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