Revision 4

Store at -20C

#4147

Cleaved Notch1 (Val1744) (D3B8) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, ChIP	H M R	Endogenous	110	Rabbit IgG	#P46531	4851

Product Usage Information

For optimal ChIP results, use 2.5 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:200
Chromatin IP	1:200

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.

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).

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 composed 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).

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.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

W: Western Blotting **IP:** Immunoprecipitation **ChIP:** Chromatin IP

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat

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