

#2745 Store at -20°C

# NEDD8 Antibody

✓ 100 µl  
(10 western blots)



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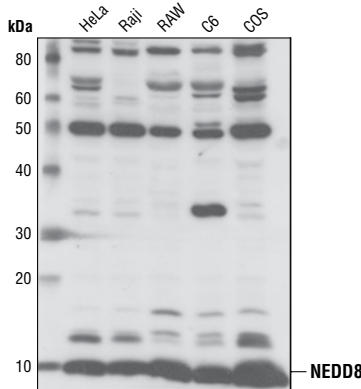
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, F, IHC-P Endogenous	H, M, R, Mk, (B, X, Z)	9 kDa	Rabbit**

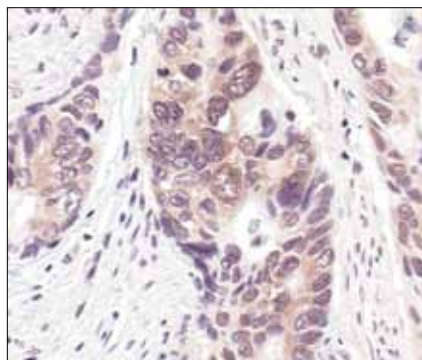
**Background:** Neural precursor cell-expressed developmentally downregulated protein 8 (NEDD8), also known as Rub1 (related to ubiquitin 1) in plants and yeast, is a member of the ubiquitin-like protein family (1,2). The covalent attachment of NEDD8 to target proteins, termed neddylation, is a reversible, multi-step process analogous to ubiquitination. NEDD8 is first synthesized in a precursor form with a carboxy-terminal extension peptide that is removed by either the UCH-L3 or NEDP1/DEN1 hydrolase protein to yield a mature NEDD8 protein (3,4). Mature NEDD8 is then covalently linked to target proteins via the carboxy-terminal glycine residue in a reaction catalyzed by the APP-BP1/Uba3 heterodimer complex and Ubc12 as the E1- and E2-like enzymes, respectively (5). An E3 ligase protein, Roc1/Rbx1, is also required for neddylation of the cullin proteins (6). Protein de-neddylation is catalyzed by a number of enzymes in the cell, including a "ubiquitin-specific" protease USP21, the NEDP1/DEN1 hydrolase and the COP9/signalosome (CSN) (7,8,9). In contrast to the ubiquitin pathway, the NEDD8 modification system acts on only a few substrates and does not appear to target proteins for degradation. Neddylation of cullin proteins activates the SCF (Skp1-Cullin-F-box) E3 ubiquitin ligase complex by promoting complex formation and enhancing the recruitment of the E2-ubiquitin intermediate (10). While NEDD8 modification of VHL is not required for ubiquitination of HIF  $\alpha$ , it is required for fibronectin matrix assembly (11). Mdm2-dependent neddylation of p53 inhibits its transcriptional activity (12).

**Specificity/Sensitivity:** This antibody detects endogenous levels of both free and conjugated NEDD8 protein. The antibody does not cross-react with other ubiquitin family members, including ubiquitin, SUMO1, SUMO2, SUMO3 and ISG15.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino acids at the amino-terminus of human NEDD8 protein. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of lysates from HeLa, Raji, RAW, C6 and COS cells using NEDD8 antibody.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma, showing cytoplasmic and nuclear localization, using NEDD8 Antibody.

Entrez-Gene ID #4738  
Swiss-Prot Acc. #Q15843

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. 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	1:25
Immunohistochemistry (Paraffin)	1:200†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Flow Cytometry	1:100

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

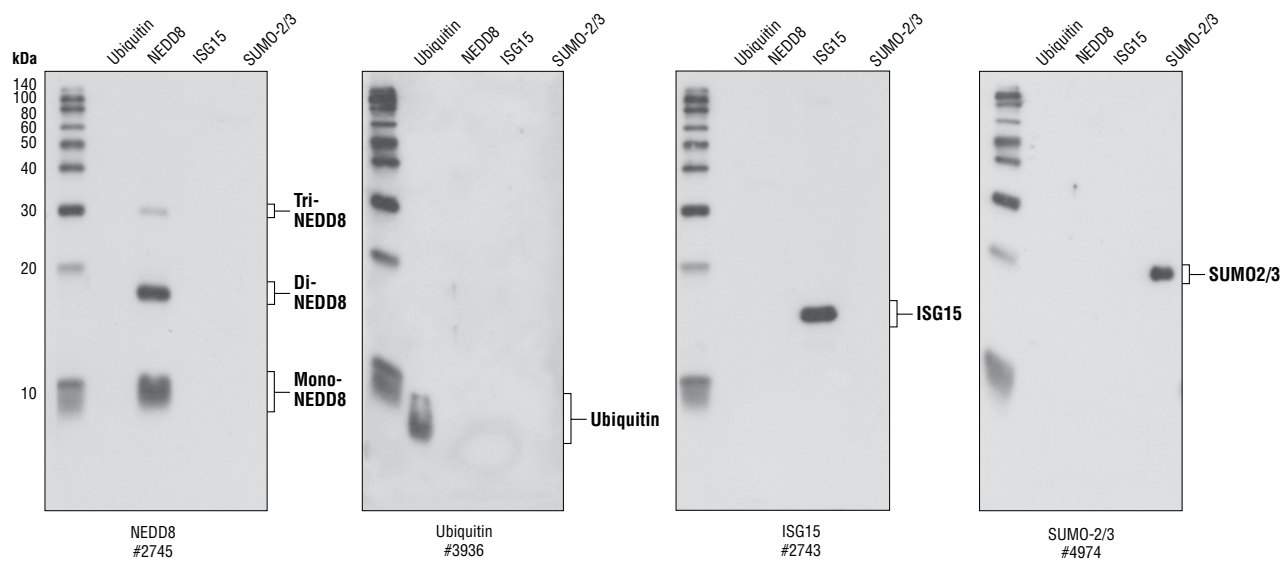
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Background References:**

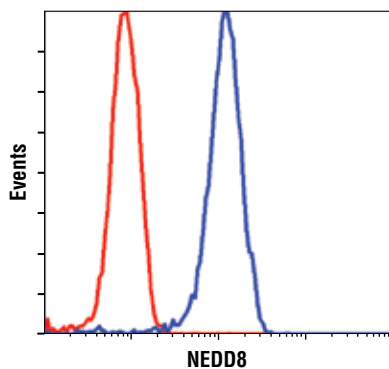
- (1) Chiba, T. and Tanaka, K. (2004) *Curr. Protein Pept. Sci.* 5, 177-84.
- (2) Schwartz, D.C. and Hochstrasser, M. (2003) *Trends Biochem. Sci.* 28, 321-8.
- (3) Wada, H. et al. (1998) *Biochem. Biophys. Res. Commun.* 251, 688-92.
- (4) Hemelaar, J. et al. (2004) *Mol. Cell Biol.* 24, 84-95.
- (5) Osaka, F. et al. (1998) *Genes Dev.* 12, 2263-8.
- (6) Kamura, T. et al. (1999) *Genes Dev.* 13, 2928-33.
- (7) Gong, L. et al. (2000) *J. Biol. Chem.* 275, 14212-6.
- (8) Mendoza, H.M. et al. (2003) *J. Biol. Chem.* 278, 25637-43.
- (9) Lyapina, S. et al. (2001) *Science* 292, 1382-5.
- (10) Kawakami, T. et al. (2001) *EMBO J.* 20, 4003-12.
- (11) Stickle, N.H. et al. (2004) *Mol. Cell Biol.* 24, 3251-61.
- (12) Xirodimas, D.P. et al. (2004) *Cell* 118, 83-97.

**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.**

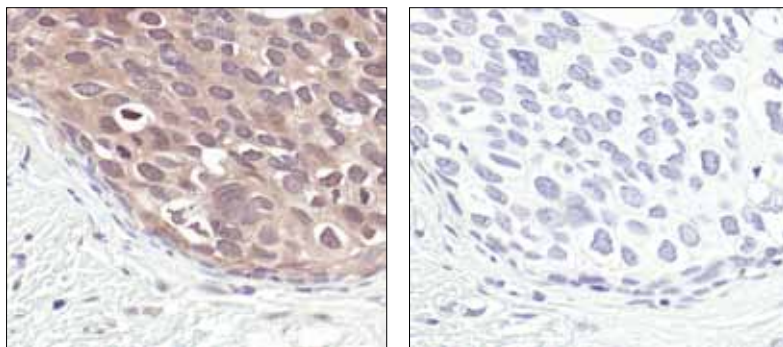
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish 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.



Western blot analysis of Ubiquitin, NEDD8, ISG15 and SUMO-2/3 recombinant proteins (5 ng each), using NEDD8, Ubiquitin, ISG15 and SUMO-2/3 Antibodies.



Flow cytometric analysis of Raji cells using NEDD8 Antibody (blue) compared to a nonspecific negative control antibody (red).



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using NEDD8 Antibody in the presence of control peptide (left) or Nedd8 Blocking Peptide #1048 (right).