

#2754 Store at -20°C

NEDD8 (19E3) Rabbit mAb

100 μl
 (10 Western mini-blot)



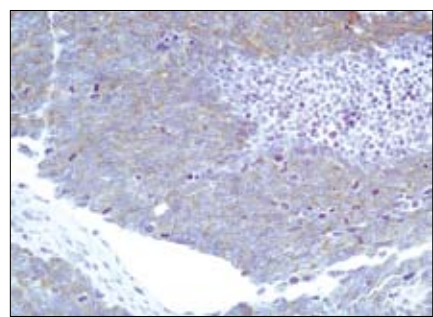
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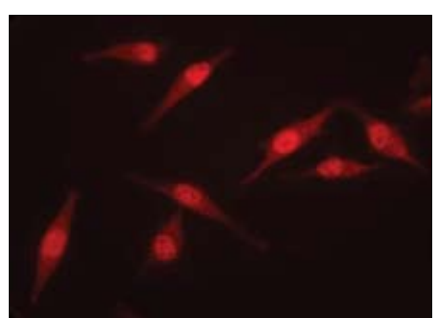
This product is for *in vitro* research use only and is not intended for use in humans or animals.
 This product is not intended for use as a therapeutic or in diagnostic procedures.

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

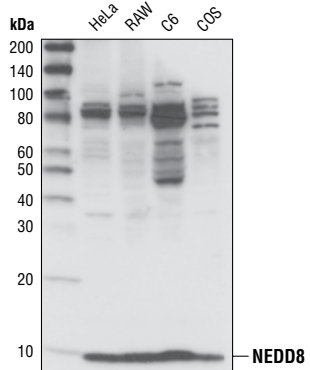
Background: Neural precursor cell-expressed developmentally downregulated protein 8 (NEDD8), also known as Rub1 (related to ubiquitin 1) in plants and yeast, is a member of the ubiquitin-like protein family (1,2). The covalent attachment of NEDD8 to target proteins, termed neddylation, is a reversible, 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 HIF1- α , it is required for fibronectin matrix assembly (11). Mdm2-dependent neddylation of p53 inhibits its transcriptional activity (12).



Immunohistochemical analysis of paraffin-embedded human lung carcinoma, using NEDD8 (19E3) Rabbit mAb.



Immunofluorescent analysis of paraformaldehyde-fixed HeLa cells, using NEDD8 (19E3) Rabbit mAb.



Western blot analysis of lysates from HeLa, RAW, C6 and COS cells, using NEDD8 (19E3) Rabbit mAb.

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

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 $\mu\text{g/ml}$ 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	1:25
IHC protocol: Unmasking buffer/Antibody diluent Citrate/TBST-5%NGS	
Immunohistochemistry (Paraffin)	1:150
Immunofluorescence (IF-IC)	1:200
Flow Cytometry	1:200

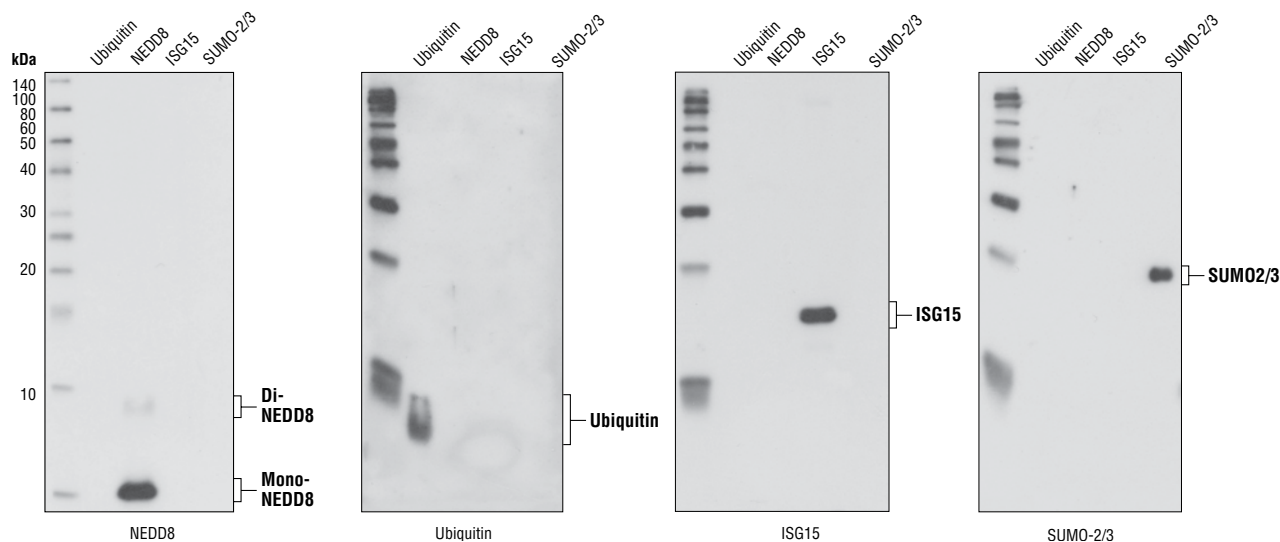
For application specific protocols please see the web page for this product at www.cellsignal.com.

- Companion Products:**
- NEDD8 Antibody #2745
 - NEDD8 Blocking Peptide #1048
 - ISG15 Antibody #2743
 - Ubiquitin (P4D1) Mouse mAb #3936
 - SUMO-1 Antibody #4930
 - SUMO-2/3 Antibody #4974
 - CUL1 Antibody #4995
 - UBC3 Antibody #4997
 - UBC3B Antibody #4996
 - Phototope[®]-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 - Anti-rabbit IgG, HRP-linked Antibody #7074
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder Detection Pack #7727
 - 20X LumiGLO[®] Reagent and 20X Peroxide #7003
- Please visit www.cellsignal.com for a complete listing of recommended companion products.**

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.

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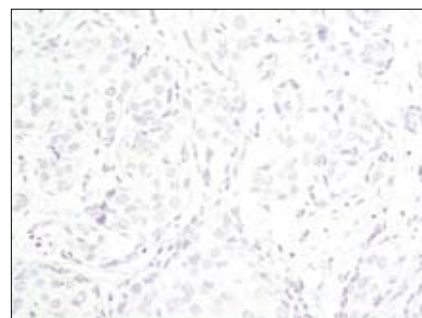
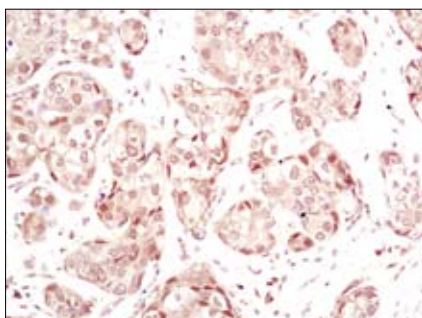
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish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



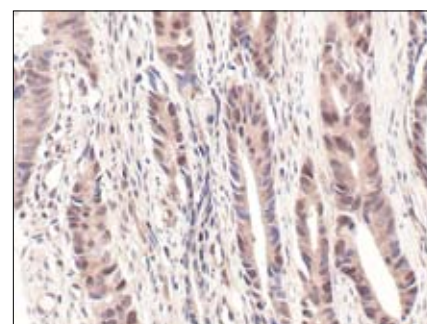
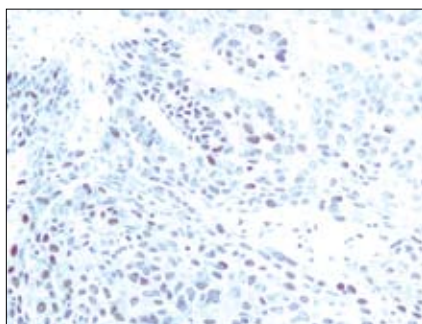
Western blot analysis of ubiquitin, NEDD8, ISG15 and SUMO-2/3 recombinant proteins (5 ng each), using NEDD8 (19E3) Rabbit mAb, Ubiquitin (P4D1) Mouse mAb #3936, ISG15 Antibody #2743 and SUMO-2/3 Antibody #4974.

Background References:

- (1) Chiba, T. and Tanaka, K. (2004) *Curr. Protein Pept. Sci.* 5, 177–184.
- (2) Schwartz, D.C. and Hochstrasser, M. (2003) *Trends Biochem. Sci.* 28, 321–328.
- (3) Wada, H. et al. (1998) *Biochem. Biophys. Res. Commun.* 251, 688–692.
- (4) Hemelaar, J. et al. (2004) *Mol. Cell Biol.* 24, 84–95.
- (5) Osaka, F. et al. (1998) *Genes Dev.* 12, 2263–2268.
- (6) Kamura, T. et al. (1999) *Genes Dev.* 13, 2928–2933.
- (7) Gong, L. et al. (2000) *J. Biol. Chem.* 275, 14212–14216.
- (8) Mendoza, H.M. et al. (2003) *J. Biol. Chem.* 278, 25637–25643.
- (9) Lyapina, S. et al. (2001) *Science* 292, 1382–1385.
- (10) Kawakami, T. et al. (2001) *EMBO J.* 20, 4003–4012.
- (11) Stickle, N.H. et al. (2004) *Mol. Cell Biol.* 24, 3251–3261.
- (12) Xirodimas, D.P. et al. (2004) *Cell* 118, 83–97.

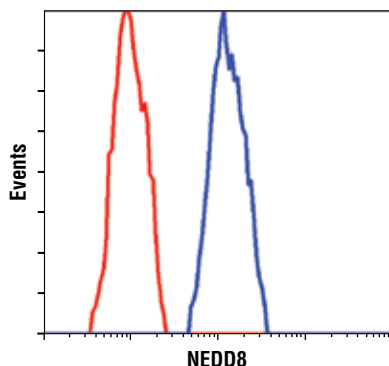


Immunohistochemical analysis of paraffin-embedded human breast carcinoma using NEDD8 (19E3) Rabbit mAb #2754 in the presence of control peptide (left) or NEDD8 Blocking Peptide (right).



Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma, using NEDD8 (19E3) Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded human colon carcinoma, using NEDD8 (19E3) Rabbit mAb.



◀ Flow cytometric analysis of untreated HeLa cells, using NEDD8 (19E3) Rabbit mAb (blue) compared to a nonspecific negative control antibody (red).