

#2740 Store at -20°C

NEDD4 Antibody

✓ 100 µl
(10 Western mini-blots)



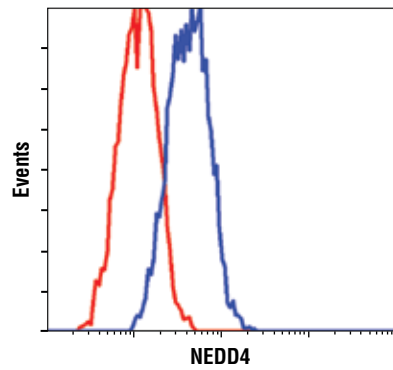
Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 08/26/08

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.	Source
W, IF-IC, F Endogenous	H, M, R, Mk	115 kDa	Rabbit**

Background: NEDD4 (neural precursor expressed, developmentally down-regulated protein 4) was originally identified as a gene that is highly expressed in the early mouse embryonic central nervous system (1). More recently, a family of NEDD4-like proteins has been defined (7 members in humans) (2). NEDD4 and NEDD4-like proteins contain multiple functional domains including a calcium-dependent phospholipid and membrane binding domain (C2 domain), two to four protein binding domains (WW domains), and an E3 ubiquitin-protein ligase domain (HECT domain). NEDD4 and NEDD4-2 have been shown to downregulate both neuronal voltage-gated Na⁺ channels (NaVs) and epithelial Na⁺ channels (ENaCs) in response to increased intracellular Na⁺ concentrations (3,4). The WW domains of NEDD4 bind to PY motifs (amino acid sequence PPXY) found in multiple NaV and ENaC proteins, and ubiquitination of these proteins, mediated by the HECT domain of NEDD4, results in their internalization and removal from the plasma membrane. Mutation of the PY motifs in ENaC proteins is associated with Liddle's Syndrome, an autosomal-dominant form of hypertension (5). In addition to targeting sodium channels, NEDD4-2 has also been shown to negatively regulate TGF-β signaling by targeting Smad2 for degradation (6). Mouse and human NEDD4 are rapidly cleaved by caspase proteins during apoptosis, although the significance of this cleavage is not clear (7).



Flow cytometric analysis of NIH/3T3 cells, using NEDD4 Antibody (blue) compared to a nonspecific negative control antibody (red).

Specificity/Sensitivity: This antibody detects endogenous levels of total NEDD4 protein. The antibody may also recognize other NEDD4-like proteins, including NEDD4L (NEDD4-2).

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to human NEDD4 protein. Antibodies are purified by protein A and peptide affinity chromatography.

Entrez-Gene ID #4734
Swiss-Prot Acc. #P46934

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:

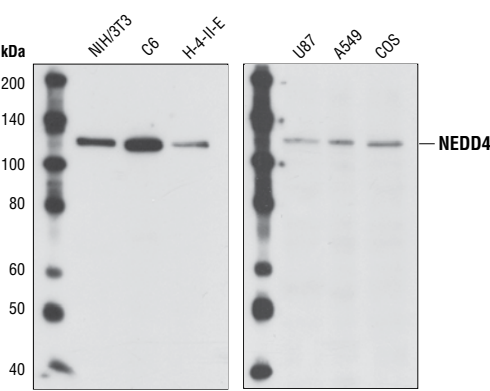
Western blotting	1:1000
Immunofluorescence (IF-IC)	1:50
Flow Cytometry	1:100

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

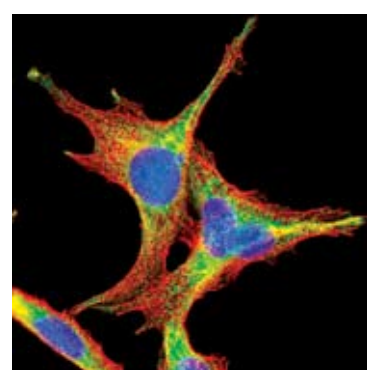
- Companion Products:**
- NEDD8 Antibody #2745
 - Phototope[®]-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 - Anti-rabbit IgG, HRP-linked Antibody #7074
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder Detection Pack #7727
 - 20X LumiGLO[®] Reagent and 20X Peroxide #7003

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

- Background References:**
- (1) Kumar, S. et al. (1992) *Biochem. Biophys. Res. Commun.* 185, 1155–61.
 - (2) Harvey, K.F. and Kumar, S. (1999) *Trends Cell Biol.* 9, 166–9.
 - (3) Dinudom, A. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 7169–73.
 - (4) Goulet, C.C. et al. (1998) *J. Biol. Chem.* 273, 30012–7.
 - (5) Staub, O. et al. (1996) *EMBO J.* 15, 2371–80.
 - (6) Kuratomi, G. et al. (2005) *Biochem. J.* 386, 461–70.
 - (7) Harvey, K.F. et al. (1998) *J. Biol. Chem.* 273, 13524–30.



Western blot analysis of lysates from NIH/3T3, C6, H-4-II-E, U87, A549 and COS cells, using NEDD4 antibody.



Confocal immunofluorescent analysis of untreated C6 cells using NEDD4 Antibody (green). Blue pseudocolor = DRAQ5[™] (DNA fluorescent dye).

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.

© 2008 Cell Signaling Technology, Inc.

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% homology.