

Phospho-NEDD4L (Ser342) (D16D6) Rabbit mAb

✓ 100 µl
(10 western blots)

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

New 10/12

For Research Use Only. Not For Use In Diagnostic Procedures.

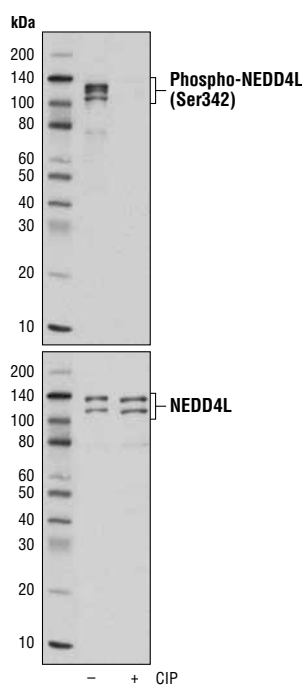
Applications W, IP Endogenous	Species Cross-Reactivity* H, M, R	Molecular Wt. 110,135 kDa	Isotype Rabbit IgG**
-------------------------------------	--------------------------------------	------------------------------	-------------------------

Background: Neural precursor expressed, developmentally down-regulated protein 4 (NEDD4) was originally identified as a gene that is highly expressed in the early mouse embryonic central nervous system (1). Subsequently, a family of NEDD4-like proteins have been defined that includes seven members in humans (2). NEDD4 and NEDD4-like (NEDD4L) 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 NEDD4L 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; ubiquitination of these proteins is mediated by the HECT domain of NEDD4 and results in their internalization and removal from the plasma membrane. Research studies have shown that 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, NEDD4L 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).

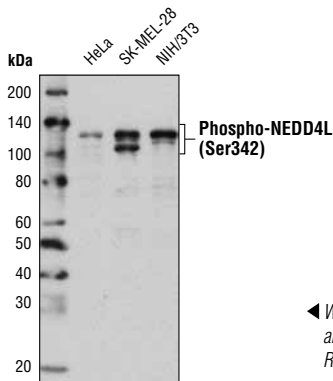
NEDD4L can be phosphorylated at Ser342 and Ser448 by several AGC kinase family members including SGK1, Akt, and PKA (8-11). Phosphorylation at these sites inhibits NEDD4L function as a suppressor of ENaCs and as a regulator of TGF-β signaling through its effects on Smad2/3 binding (8-11).

Specificity/Sensitivity: Phospho-NEDD4L (Ser342) (D16D6) Rabbit mAb recognizes endogenous levels of NEDD4L protein only when phosphorylated at Ser342.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser342 of human NEDD4L protein.



Western blot analysis of extracts from SK-MEL-28 cells, untreated (-) or CIP-treated (+), using Phospho-NEDD4L (Ser342) (D16D6) Rabbit mAb (upper) or NEDD4L Antibody #4013 (lower).



◀ Western blot analysis of extracts from HeLa, SK-MEL-28, and NIH/3T3 cells using Phospho-NEDD4L (Ser342) (D16D6) Rabbit mAb.

Entrez-Gene ID #23327
Swiss-Prot Acc. #Q96PU5

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	1:100

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

Please visit www.cellsignaling.com for a complete listing of recommended complementary 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.
- (8) Gao, S. et al. (2009) *Mol Cell* 36, 457-68.
- (9) Lee, I.H. et al. (2007) *J Biol Chem* 282, 29866-73.
- (10) Snyder, P.M. et al. (2004) *J Biol Chem* 279, 45753-8.
- (11) Debonneville, C. et al. (2001) *EMBO J* 20, 7052-9.

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.