

NEDD8 [untagged]

Modifier

Alternate Names: Neural precursor cell expressed, developmentally down regulated 8, Ubiquitin like protein NEDD8

Cat. No. 60-0009-500

Lot. No. 30167

Quantity: 500 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

Background

The enzymes of the NEDDylation pathway play a pivotal role in the activation of the largest class of ubiquitin E3 ligases called Cullin RING Ligases (CRLs). Akin to ubiquitylation three classes of enzymes are involved in the process of mammalian NEDDylation; E1 activating enzyme (APP-BP1/Uba3 heterodimer), E2 conjugating enzymes (UBE2F or UBE2M) and E3 ligases (Meyer-Schaller, *et al.*, 2009) including the Domain Containing Like Protein1 (DCNL1) and Ring Box 1 (RBX1) heterodimer (Huang *et al.*, 2011; Morimoto *et al.*, 2003). Neural Precursor Cell Expressed, Developmentally Downregulated 8 (NEDD8) is a member of the ubiquitin like modifiers and the human gene was first described by (Kamitani, *et al.*, 1997). The heterodimeric E1, APP-BP1/Uba3 forms a complex *in vitro* and a thioester linkage with NEDD8 (Osaka *et al.*, 1998). NEDD8 has also been co-crystallised with APP-BP1 and ATP (Walden *et al.*, 2003). The structure consists of an E1-specific domain organised around a catalytic cysteine and a domain involved in E2 recognition which coordinates protein binding and drives the E1's reactions. This ATP-dependent activation of NEDD8 enables its transfer via a transthiolation reaction to either of the NEDD8 E2 conjugating enzymes UBE2F or UBE2M. Subsequently the NEDD8 is conjugated onto the cullin subunit of

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 500 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~8.6 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70°C; aliquot as required

Protein Sequence:

**MLIKVKTLTGKEIEIDIEPTDKVERIK
ERVEEKEGIPPOQRLLIYSGKQMNDEK
TAADYKILGGSVLHLVLALRGG**

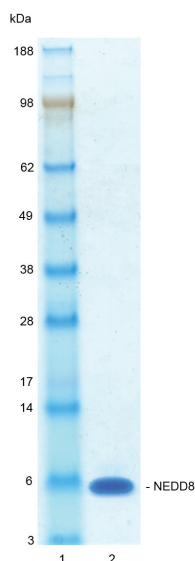
NEDD8 (regular text): Start **bold italics** (amino acid residues 1-76)

Accession number: NP_006147.1

Quality Assurance

Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg NEDD8



Protein Identification:

Confirmed by mass spectrometry.

E2 thioester NEDD8 loading assay. The activity of NEDD8 was validated by loading E1 APP-BP1/Uba3 activated NEDD8 onto the active cysteine of the His-UBE2M E2 enzyme via a transthiolation reaction. Incubation of the APP-BP1/Uba3 and His-UBE2M enzymes in the presence of NEDD8 and ATP at 30°C was compared at two time points, T₀ and T₁₀ minutes. Sensitivity of the NEDD8/His-UBE2M thioester bond to the reducing agent DTT was confirmed.



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Lot-specific COA version tracker: v1.0.0

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the CRL. This has been shown to occur through N-terminal acetylation of the E2 conjugating enzyme Ube2M and burial of the N-acetyl- methionine of Ube2M into a hydrophobic pocket of Defective in Cullin Neddylation 1 (DCNL1) which promotes an E3 dependent Neddylation of Cullin1 (Scott *et al.*, 2011).

References:

Huang G, Kaufman AJ, Ramanathan Y, Singh B (2011) SCCRO (DCUN1D1) promotes nuclear translocation and assembly of the neddylation E3 complex, *J Biol Chem* **286**, 10297-10304.

Kamitani T, Kito K, Nguyen HP, Yeh ET (1997) Characterization of NEDD8, a developmentally down-regulated ubiquitin-like protein, *J Biol Chem* **272**, 28557-28562.

Meyer-Schaller N, Chou YC, Sumara I, Martin DD, Kurz T, Katheder N, Hofmann K, Berthiaume LG, Sicheri F, Peter M (2009) The human Dcn1-like protein DCNL3 promotes Cul3 neddylation at membranes, *Proc Natl Acad Sci U S A* **106**, 12365-12370.

Morimoto M, Nishida T, Nagayama Y, Yasuda H (2003) Nedd8-modification of Cul1 is promoted by Roc1 as a Nedd8-E3 ligase and regulates its stability, *Biochem Biophys Res Commun* **301**, 392-398.

Walden H, Podgorski, MS and Schulman, BA (2003) Insights into the ubiquitin transfer cascade from the structure of the activating enzyme for NEDD8, *Nature* **422**, 330-334.

Scott DC, Monda JK, Bennett EJ, Harper JW, Schulman BA (2011) N-terminal acetylation acts as an avidity enhancer within an interconnected multiprotein complex, *Science* **334**, 674-678.



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