APP-BP1 [untagged] / UBA3 [untagged]

E1 Activating Enzyme

Alternate Names: NAE1, HPP1

Cat. No. **61-0006-010**
Lot. No. **30079**

Quantity: 10 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 (UBE2M or UBE2F) and E3 ligases (Meyer-Schaller *et al.* 2009) including the Domain Containing Like Protein 1 (DCNL1) and Ring Box 1 (RBX1) heterodimer (Morimoto *et al.* 2003; Huang *et al.* 2011). The APP-BP1/UBA3 heterodimer is a member of the NEDD8 E1-activating enzyme family and cloning of the human genes coding for these proteins were first described by Chow *et al.* (1996) and Osaka *et al.* (1998). The APP-BP1 (Amyloid Precursor Protein Binding Protein 1) gene has been mapped to 16q22 by high resolution fluorescence *in situ* hybridization (Chow *et al.* 1996). APP-B1 is the regulatory subunit of the E1 whose catalytic partner is UBA3. The two proteins form a complex *in vitro* and a thioester linkage with NEDD8 suggesting that the APP-BP1/UBA3 complex functions as an E1-like enzyme for the activation of NEDD8 (Osaka *et al.* 1998). The heterodimeric structure of APP1-BP1/UBA3 has been determined through co-crystallization with NEDD8 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 UBE2M or UBE2F. Subsequently the NEDD8 is conjugated onto the cullin subunit of the CRL. NEDDylation of CRLs trigger a structural change within the C-terminus of the CRL E3 complex which is necessary for the efficient ubiquitylation of its

Continued on page 2

Physical Characteristics

Species: human

Source: Insect sf21

Quantity: 10 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: APP-BP1 = 60.46 kDa
UBA3 = 49.35 kDa

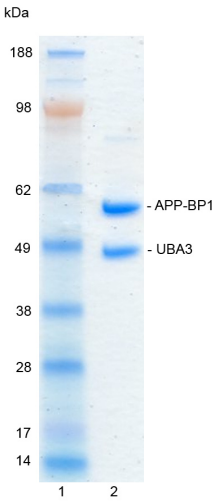
Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequences: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg APP-BP1/UBA3



Protein Identification:
Confirmed by mass spectrometry.

E1 Thioester NEDD8 Loading Assay:
The activity of APP-BP1/UBA3 was validated by loading NEDD8 onto the active cysteine of APP-BP1/UBA3. Incubation of the APP-BP1/UBA3 enzyme in the presence of NEDD8 and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the NEDD8 / APP-BP1/UBA3 thioester bond to the reducing agent DTT was confirmed.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

APP-BP1 [untagged] / UBA3 [untagged]

E1 Activating Enzyme

Alternate Names: NAE1, HPP1

Cat. No.	**61-0006-010**	Quantity:	10 µg
Lot. No.	**30079**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

substrates (Duda *et al.* 2008). Substrates of the CRLs play important roles in pathways controlling tumour cell growth. Thus a potent and selective inhibitor (MLN4924) of APP-BP1/UBA3 which disrupts CRL mediated protein turnover has been developed (Bruzzese *et al.* 2012). Treatment of human tumour cells *in vitro* with MLN4924 leads to apoptotic death by the de-regulation of S-phase DNA synthesis (Soucy *et al.* 2009). Senescence was identified as another mechanism of action for MLN4924 in suppressing tumour cell growth through the inhibition of SKP1-Cullin-F-box proteins (SCF) E3 ubiquitin ligases and accumulation of p21 in tumour cell lines (Jia *et al.* 2011). MLN4924 is now undergoing clinical trials for the treatment of various hematological malignancies.

References:

Bruzzese FJ, Milhollen MA, Gavin JM, Josephine HR, Brownell JE (2012) Identification and application of NEDD8 E1 inhibitors. *Methods Mol Biol* **832**, 577-588.

Chow N, Korenberg JR, Chen XN, Neve RL (1996) APP-BP1, a novel protein that binds to the carboxyl-terminal region of the amyloid precursor protein. *J Biol Chem* **271**, 11339-11346.

Duda DM, Borg LA, Scott DC, Hunt HW, Hammel M, et al. (2008) Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. *Cell* **134**, 995-1006.

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.

Jia L, Li H, Sun Y (2011) Induction of p21-dependent senescence by an NAE inhibitor, MLN4924, as a mechanism of growth suppression. *Neoplasia* **13**, 561-569.

Meyer-Schaller N, Chou YC, Sumara I, Martin DD, Kurz T, et al. (2009) The human Dcn1-like protein DCNL3 promotes Cul3 neddylation at membranes. *Proc Natl Acad Sci USA* **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.

Osaka F, Kawasaki H, Aida N, Saeki M, Chiba T, et al. (1998) A new NEDD8-ligating system for cullin-4A. *Genes Dev* **12**, 2263-2268.

Soucy TA, Smith PG, Milhollen MA, Berger AJ, Gavin JM, et al. (2009) An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* **458**, 732-736.

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

Physical Characteristics

Continued from page 1

APP-BP1 Protein Sequence:

GGS***M***AQLGKLLKEQKYDRQLRLWGDHGQEALE
SAHVCLINATATGTEILKNLVLPGIGSFTI
IDGNQVSGEDAGNNFFLQRSSIGKNRAEAAME
FLQELNSDVSGSFVEESPENLLDNDPSFFCR
FTVVVATQLPESTSLRLADVLWNSQIPLLI
CRTYGLVGYMRIIIKEHPVIESHPDNALEDL
RLDKPFPELREHFQSYDLDHMEKKDHSHTP
WIVIIAKYLAQWYSETNGRIPKTYKEKED
FRDLIRQGILKNENGAPEDEENFEEAIKNVN
TALNTTQIPSSIEDIFNDDRCINITKQTPS
FWILARALKEFVAKEGQGNLPVRGTIPD
MIADSGKYIKLQNVYREKAKKDAAAVGNH
VAKLLQSIGQAPESISEKELKLLCSNSAFLRV
VRCRSLAEEYGLDTINKDEIISSMDNPDNEIV
LYLMLRAVDRFHKQQGRYPGVSNYQVEEDIG
KLKSCLTGFLQEYGLSVMVKDDYVHEFCRY
GAAEPHTIAAFLGGAAAQEVIKIITKQFVIF
NNTYIYSGMSQTSATFQL

The residues underlined remain after cleavage and removal of the purification tag.
APP-BP1 (regular text): Start ***bold italics*** (amino acid residues 3-536)
Accession number: NP_003896

UBA3 Protein Sequence:

MAVDGGCGDTGDWEGRWNHVKKFLERSGPFTHP
DFEPSTESLQFLLDTCKVLVIGAGGLGCELLKN
LALSGFRQIHVIDMDTIDVSNLNRQFLFRPKDI
GRPKAEVAAEFLNDRVPNCNVVPHFNKIQDF
NDTFYRQFHIIVCGLDSIIARRWINGMLISLL
NYEDGVLDPSSIVPLIDGGTEGFKGNARVILPG
MTACIECTLELYPPQVNFPMCTIASMPRLPEH
CIEYVRMLQWPKEQPFGEGVPLDGDDPEHIQ
WIFQKSLERASQYNIRGVTYRLTQGVVKRII
PAVASTNAVIAAVCATEVFKIATSAYIPLNNYL
VFNDVDGLYTYTFEAERKENCPACSQLPQNIQF
SPSAKLQEVLDYLTNSASLQMKSPAITATLEG
KNRTLYLQSVTSIEERTRPNLSKTLKEL
GLVDGQELAVADVTTPQTVLFKLHFTS

UBA3 (regular text): Start ***bold italics*** (amino acid residues 22-463)
Accession number: NP_003959.3

To purify the APP-BP1/UBA3 heterodimer the genes for these two proteins were co-expressed using the baculovirus/insect cell expression system (APP-BP1 was tagged with a protease cleavable proprietary tag) and a proprietary resin was used to capture the tagged APP-BP1/UBA3 heterodimer. 6His-tagged protease was then used to cleave the tag releasing the APP-BP1/UBA3. This eluate was then incubated with nickel and the proprietary resins to remove the protease and any uncleaved APP-BP1 respectively. The non-bound fraction containing APP-BP1/UBA3 heterodimer was dialysed into the storage buffer. Based on the SDS-PAGE analysis it is likely that the majority if not all of the species in the preparation is APP-BP1/UBA3 heterodimer with little if any free APP-BP1.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0