Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Dac-APP-BP1 / UBA3

Enzyme description:- Dac-APP-BP1 / UBA3

Clone number:- DU21784 (SC21784)

Source:- human recombinant

Tag:- N-terminal Dac- on APP-BP1

Purification method:- Ampicillin-Sepharose

Expression level:- 15mg/L

Calculated molecular mass for Dac-APPBP1:-

Monoisotopic	90412 Da
Average Mass	90466 Da

[cysteines reduced, methionines have not been oxidised]

Calculated molecular mass for UBA3:-

Monoisotopic	49311 Da
Average Mass	49341 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI for Dac-APPBP1:- 5.70

Theoretical pI for UBA3:- 5.39

Purity:- 90%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80°C

Assay:-

Loading of NEDD8 to itself and/or UBE2M or UBE2F

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Clone Data Sheets

Protein name Dac-APP-BP1 / UBA3

Protein	Dac-APP-BP1
Synonyms	NAE1; ULA1 human; NEDD8-activating enzyme E1 regulatory subunit
Clone Number	DU21784 (SC21784)
Species	Human
Accession Number	NP_003959
Tags	Dac- N-terminal of APPBP1
Aminoacid sequence of the expressed protein	**Dac-APP-BP1:** MSAIPGVPQIDAESYILIDYNSGKVLAEQNADVRRDPASLTKMMTS YVIGQAMKAGKFKETDLVTIGNDAWATGNPVFKGSSLMFLKPGMQV PVSQLIRGINLQSGNDACVAMADFAAGSQDAFVGLMNSYVNALGLK NTHFQTVHGLDADGQYSSARDMALIGQALIRDVPNEYSIYKEKEFT FNGIRQLNRNGLLWDNSLNVDGIKTGHTDKAGYNLVASATEGQMRL ISAVMGGRTFKGREAESKKLLTWGFRFFETVNPLVPRGSENLYFQG GS**MAQLGKLLKEQKYDRQLRLWGDHGQEALESAHVCLINATATGTE** **ILKNLVLPGIGSFTIIDGNQVSGEDAGNNFFLQRSSIGKNRAEAAM** **EFLQELNSDVSGSFVEESPENLLDNDPSFFCRFTVVVATQLPESTS** **LRLADVLWNSQIPLLICRTYGLVGYMRIIIKEHPVIESHPDNALED** **LRLDKPFPELREHFQSYDLDHMEKKDHSHTPWIVIIAKYLAQWYSE** **TNGRIPKTYKEKEDFRDLIRQGILKNENGAPEDEENFEEAIKNVNT** **ALNTTQIPSSIEDIFNDDRCINITKQTPSFWILARALKEFVAKEGQ** **GNLPVRGTIPDMIADSGKYIKLQNVYREKAKKDAAAVGNHVAKLLQ** **SIGQAPESISEKELKLLCSNSAFLRVVRCRSLAEEYGLDTINKDEI** **ISSMDNPDNEIVLYLMLRAVDRFHKQQGRYPGVSNYQVEEDIGKLK** **SCLTGFLQEYGLSVMVKDDYVHEFCRYGAAEPHTIAAFLGGAAAQE** **VIKIITKQFVIFNNTYIYSGMSQTSATFQL**
Native sequence	residues 1- 534 = full length APP-BP1 in bold
Protease cleavage	TEV underlined
Cloning sites	BamHI HindIII

DNA sequence of insert

APP-BP1:

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GGATCCATGGCGCAGCTGGGAAAGCTGCTCAAGGAGCAGAAGTACGACCG
GCAGCTGAGGTTGTGGGGTGATCATGGGCAAGAGGCTTTAGAATCTGCTC
ATGTTTGCCTAATAAATGCAACAGCCACAGGAACTGAAATTCTTAAAAAC
TTGGTACTACCAGGTATTGGTTCGTTTACAATTATTGATGGAAATCAGGT
CAGCGGAGAAGATGCTGGAAACAATTTCTTCCTTCAAAGAAGCAGTATCG
GCAAGAACCGAGCTGAAGCTGCCATGGAATTCTTACAAGAATTAAATAGC
GATGTCTCTGGAAGTTTTGTGGAAGAGAGTCCAGAAAACCTTCTAGACAA
TGATCCCTCATTTTTCTGTAGGTTTACTGTTGTAGTTGCAACTCAGCTTC
CTGAAAGCACTTCACTACGCTTAGCAGATGTCCTCTGGAATTCCCAGATT
CCTCTTTTGATCTGTAGGACATATGGACTAGTTGGTTATATGAGGATCAT
TATAAAAGAACATCCAGTAATAGAATCTCATCCAGATAATGCATTAGAGG
ATCTACGACTAGATAAGCCATTTCCTGAACTGAGAGAACATTTTCAGTCC
TATGATTTGGATCATATGGAAAAAAAGGACCACAGTCATACTCCATGGAT
TGTGATCATAGCTAAATATTTAGCACAGTGGTATAGTGAAACAAATGGAC
GAATACCTAAAACGTATAAAGAAAAAGAGGACTTCAGAGATTTGATTAGA
CAAGGAATTCTAAAAAATGAAAATGGGGCTCCAGAAGATGAAGAGAATTT
TGAAGAAGCTATTAAAAATGTGAACACAGCACTAAATACAACTCAGATCC
CAAGCAGTATTGAAGATATATTTAATGATGATCGCTGCATAAATATCACC
AAACAGACTCCATCATTTTGGATTTTAGCTCGTGCCTTAAAGGAATTTGT
GGCCAAAGAGGGTCAAGGAAATTTACCTGTTCGAGGCACAATTCCTGATA
TGATTGCAGATTCAGGCAAATATATAAAACTGCAAAACGTTTACCGTGAA
AAAGCAAAGAAAGATGCTGCCGCTGTGGGTAATCATGTTGCCAAATTGCT
GCAGTCCATTGGCCAGGCACCAGAGTCCATTTCAGAGAAAGAATTAAAAT
TACTCTGCAGCAATTCTGCATTTCTTCGAGTGGTAAGATGTCGATCCTTA
GCTGAAGAATATGGTTTGGATACAATTAACAAGGATGAAATTATTTCTAG
CATGGACAATCCAGATAATGAAATAGTGTTGTACTTAATGTTACGGGCTG
TTGATAGATTTCATAAACAACAGGGTAGATATCCAGGAGTATCTAACTAT
CAAGTTGAAGAAGATATAGGAAAGTTGAAGTCTTGTCTCACTGGCTTCCT
TCAGGAATATGGTTTATCTGTAATGGTGAAAGATGATTATGTCCACGAAT
TTTGCCGATATGGAGCTGCTGAGCCACATACCATTGCTGCATTCTTGGGG
GGAGCTGCTGCTCAAGAGGTCATCAAAATAATCACCAAACAATTTGTAAT
TTTTAATAATACTTACATTTACAGTGGCATGTCACAAACTTCAGCAACTT
TCCAGTTGTAGAAGCTT
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Protein	**UBA3**
Synonyms	NAE2; NEDD8-activating enzyme E1 catalytic subunit
Clone Number	DU21784 (SC21784)
Species	Human
Accession Number	NP_003896
Tags	n/a
Aminoacid sequence of the expressed protein	**MAVDGGCGDTGDWEGRWNHVKKFLERSGPFTHPDFEPSTESLQFLL** **DTCKVLVIGAGGLGCELLKNLALSGFRQIHVIDMDTIDVSNLNRQF** **LFRPKDIGRPKAEVAAEFLNDRVPNCNVVPHFNKIQDFNDTFYRQF** **HIIVCGLDSIIARRWINGMLISLLNYEDGVLDPSSIVPLIDGGTEG** **FKGNARVILPGMTACIECTLELYPPQVNFPMCTIASMPRLPEHCIE** **YVRMLQWPKEQPFGEGVPLDGDDPEHIQWIFQKSLERASQYNIRGV** **TYRLTQGVVKRIIPAVASTNAVIAAVCATEVFKIATSAYIPLNNYL** **VFNDVDGLYTYTFEAERKENCPACSQLPQNIQFSPSAKLQEVLDYL** **TNSASLQMKSPAITATLEGKNRTLYLQSVTSIEERTRPNLSKTLKE** **LGLVDGQELAVADVTTPQTVLFKLHFTS**
Native sequence	residues 1 – 442 = full length in bold
Protease cleavage	N/A
Cloning sites	Nhe1 / kpn1
DNA sequence of insert	**UBA3:** gctagcatggcggatggctgttgatggtgggtgtggggacactggagact gggaaggtcgctggaaccatgtaaagaagttcctcgagcgatctggaccc ttcacacaccctgatttcgaaccgagcactgaatctctccagttcttgtt agatacatgtaaagttctagtcattggagctggcggcttaggatgtgagc tcctgaaaaatctggccttgtctggttttagacagattcatgttatagat atggacactatagatgtttccaatctaaataggcagtttttatttaggcc taaagatattggaagacctaaggctgaagttgctgcagaatttctaaatg acagagttcctaattgcaatgtagttccacatttcaacaagattcaagat tttaacgacactttctatcgacaatttcatattattgtatgtggactgga ctctatcatcgccagaagatggataaatggcatgctgatatctcttctaa attatgaagatggtgtcttagatccaagctccattgtccctttgatagat ggggggacagaaggttttaaaggaaatgcccgggtgattctgcctggaat gactgcttgtatcgaatgcacgctggaactttatccaccacaggttaatt ttcccatgtgcaccattgcatctatgcccaggctaccagaacactgtatt gagtatgtaaggatgttgcagtggcctaaggagcagccttttggagaagg ggttccattagatggagatgatcctgaacatatacaatggattttccaaa aatccctagagagagcatcacaatataatattaggggtgttacgtatagg ctcactcaaggggtagtaaaaagaatcattcctgcagtagcttccacaaa tgcagtcattgcagctgtgtgtgccactgaggtttttaaaatagccacaa gtgcatacattcccttgaataattacttggtgtttaatgatgtagatggg ctgtatacatacacatttgaagcagaaagaaaggaaaactgcccagcttg tagccagcttcctcaaaatattcagttttctccatcagctaaactacagg aggttttggattatctaaccaatagtgcttctctgcaaatgaaatctcca gccatcacagccaccctagagggaaaaaatagaacactttacttacagtc ggtaacctctattgaagaacgaacaaggccaaatctctccaaaacattga aagaattggggcttgttgatggacaagaactggcggttgctgatgtcacc accccacagactgtactattcaaacttcattttacttcttaaggtacc