

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active VPS34 [2 - 887] and VPS15 [1 – 1358]

Enzyme description:- VPS34 [2 - 887] and VPS15 [1- 1358]

Clone number:- DU 8692

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6) and HA for VPS34
No tag for VPS15

Purification method:- Ni²⁺-NTA agarose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic 106, 453.88 daltons [VPS34] and 153, 006.59 [VPS15]
Average Mass 106, 521.77 daltons [VPS34] and 153, 103.47 [VPS15]
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.05 [VPS34] and 6.74 [VPS15]

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay:- ADP Glo

Assay buffer:-

20 mM Tris-HCl, 67 mM KCl, 1 mM EDTA, 1 mM DTT, 0.05 mg/ml BSA, 10 mM MgCl₂

Substrate:-

PI Final concentration: 0.04 mM

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Clone Data Sheet

VPS34 [2 – 887] and VPS15 [1 - 1358]

Protein VPS34 [2 - 887] and VPS15 [1 1358]
Clone number DU 8692
Species Human
Accession number NM_002647 [VPS34] and NM_014602 [VPS15]
Tags VPS34 - N-terminal His(6) and HA (YPYDVPDYA)
VPS15 - untagged

**Baculovirus
expressed VPS34
protein**

MSYYHHHHHDYDIPTTENLYFQ~~G~~AMDPEFATMYPYDVPDYAGEAEK~~F~~H
YIYSCDL~~D~~INVQLKIGSLE~~G~~KREQ~~K~~SYKAVLEDPMLKFSGLYQETCSD~~L~~
YVTCQVFAEGKPLALPVRTSYKAFSTRWNWNEWLKL~~P~~VKYPDLPRNAQ~~V~~
ALT~~I~~WDVYGP~~G~~KAVPVGGTTVSLFGKYGMFRQGMHDLK~~V~~WPNVEADGSE
PTKTPGR~~T~~SSTLSE~~D~~QMSRLAKLTKAHRQGHMV~~K~~VDWLDRLTFREIEMI
NESEKRSSNFMYLMVEFRCVKCDDKEYGIVYYEKDGE~~S~~SPILTSFELV
KVPDPQMSMENLVESKHHKLARSLRSGPSDHDLPNAATRD~~Q~~LNIIVSY
PPTKQLTYEEQDLVWKFRYYLTNQE~~K~~ALTKFLKCVNWDLPQEAKQALEL
LGKWKPM~~D~~VEDSLELLSSH~~Y~~TNPTVRRYAVARLRQADDEDLLMYLLQ~~L~~V
QALKYENFDDIKNGLEPTKKDSQSSVSE~~N~~SVNSGINS~~A~~EIDSSQIITSP
LPSVSSPP~~P~~ASKTKEVPDGENLEQDLCTFLISRACKNSTLAN~~Y~~LYWYVI
VECEDQDTQQRDPKTHEMYLNMRRFSQALLKGD~~K~~SVRVMRSLLAAQ~~O~~T
FVDRLVHLMKAVQRESGNRKKKNERLQALLGDNEKMNLS~~D~~VELIPLPLE
PQVKIRGIIPETATL~~F~~KSALMPAQLFFKTEDGGKYPVIFKHGDDLRQ~~D~~
LILQII~~S~~MDKLLRKENLDLKLTPYKVLATSTKHGFMQFIQSVPVAEVL
DTEGSIQNFFRKYAPSENGPNGISAEVMDTYVKSCAGYCVITYILGVGD
RHLNLLLTKTGKLFHIDFGYILGRDPKPLPPM~~K~~LNKEMVEGMGGTQS
EQYQEFRKQCYTAF~~L~~HLLRRYSNLILNLFSLMVDANIPDIALEPDKTVKK
VQDKFRLDLSDEEAVHYMQSLIDESVHALFAAVEQIHKFAQYWRK

Native sequence Amino acids G2 – K887 (end) of human VPS34.
Residue G43 of the fusion protein is equivalent to G2 of the native
enzyme. The His(6) tag is located at residues 5 – 10 and the HA tag is
located at residues 34 – 42.

Protease cleavage rTEV (ENLYFQG) residues 18 – 24

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Cloning sites

RsrII/SalI and *NheI/SalI* into *NheI/XhoI* of pFastBac Dual

MGNQLAGIAPSQILSVESYFSDIHDFEYDKSLGSTRFFKVARAKHREGL
VVKVFAIQDPTLPLTSYKQELEELKIRLNSAQNCLPFQKASEKASEKA
AMLFROYVRDNLVDRISTRPFLNNIEKRWIAFQILTAVDQAHKSGVRHG
DIKTENVMVTSWNWVLLTDFASFKPTYLPEDNPADFNFFDTSRRRTC
IAPERFVDGGMFATELEYMRDPSTPLVDLNSNQTRGELKRAMDIFSAG
CVIAELFTEGVPLFDLSQLLAYRNGHFFPEQVLNKIEDHSIRELVTQMI
HREPDKRLEAEDYLKQQRGNAPPEIFYTFLOPYMAQFAKETFLSADERI
LVIRKDLGNI IHNLCGHDLPEKAEGEPKENGLVILVSVITSCLOTLKYC
DSKLALELILHLAPRLSVEILLDRITPYLLHFSNDSVPRVRAEALRTL
TKVLALVKEVPRNDINIYPEYILPGIAHLAQDDATIVRLAYAENIALLA
ETALRFLELVQLKNLNMENDPNNEEIDEVTHPNGNYDTELOALHEMVQQ
KVVTLSDPENIVKQTLMENGITRLCVFFGRQKANDVLLSHMITFLNDK
NDWHLRGAFFDSIVGVAAYVGWQSSSILKPLLQOGLSDAEFVIVKALY
ALTCMCQLGLLQKPHVYEFASDIAPFLCHPNLWIRYGAVGFITVVARQI
STADVYCKLMPYLDPYITQPIIQIERKLVLLSVLKEPVSRISFDYALRS
KDITSLFRHLHMRQKRRNGSLPDCPPPEDPAIAQLLKKLLSQGMTEEEE
DKLLALKDFMMKSNKAKANIVDQSHLHDSSQKGVIDLALGITGRQVDL
VKTQEPDDKRARKHVQDSNVNEEWKSMFGSLDPPNMPQALPKGSDQE
VIQTGKPPRESSAGICVPLSTSSQVPEVTTVQNKKPVIPVLSSTILPS
TYQIRITTCKTELQQLIQKREQCNAERIAKQMMENA EWESKPPPPGWR
PKGLLV AHLHEHKS AVNRIRVSDEHSLFATCSNDGTVKIWNSQKMEGKT
TTTRSILTYSRIGGRVKTTLFCQGSYLAIASDNGAVQLLGI EASKLPK
SPKIHPLQSRILDQKEDGCVVDMHFN SGAQSVLAYATVNGSLVGWDLR
SSSNAWTLKHD LKSGLITSF AVDIHQWCWLCIGTSSGTMACWDMRFQLPI
SSHCHPSRARI RRLSMHPLYQSWVIAAVQGNNEVSMWDMETGDRRFTLW
ASSAPPLSELQSPHSVHGIYCSPADGNPILLTAGSDMKIRFWDLAYPE
RSYVVAGSTSSPSVSYRKIIEGTEVVQEI QNKQKVGPSDDT PRRGPES
LPVGHHDIIITDVATFQTTQGFIVTASRDGIVKVVK

Native sequence

Amino acids M1 – K1358 (end) of human VPS15.

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Nucleotide of sequence of VPS34 insert

gaattcgccaccatgtaccatagatgtgccagattacgccgggGAAG
CAGAGAAGTTTCACTACATCTATAGTTGTGACCTGGATATCAACGTCCA
GCTTAAGATAGGAAGCTTGGAAGGGAAGAGAGAACAAAAGAGTTATAAA
GCTGTCTGGAAGACCCAATGTTGAAGTTCTCAGGACTATATCAAGAGA
CATGCTCTGATCTTTATGTTACTTGTCAAGTTTTTGCAGAAGGGAAGCC
TTTGGCCTTGCCAGTGAGAACATCCTACAAAGCATTTAGTACAAGATGG
AACTGGAATGAATGGCTGAAACTACCAGTAAAATACCCTGACCTGCCCA
GGAATGCCAAGTGGCCCTCACCATATGGGATGTGTATGGTCCCGGAAA
AGCAGTGCCTGTAGGAGGAACAACGGTTTTCGCTCTTTGGAAAATACGGC
ATGTTTTCGCCAAGGGATGCATGACTTGAAAGTCTGGCCTAATGTAGAAG
CAGATGGATCAGAACCCACAAAACCTCCTGGCAGAACAAGTAGCACTCT
CTCAGAAGATCAGATGAGCCGTCTTGCCAAGCTCACCAAAGCTCATCGA
CAAGGACACATGGTGAAAGTAGATTGGCTGGATAGATTGACATTTAGAG
AAATAGAAATGATAAATGAGAGTGAAAAACGAAGTTCTAATTTTCATGTA
CCTGATGGTTGAATTTTCGATGTGTCAAGTGTGATGATAAGGAATATGGT
ATTGTTTATTATGAAAAGGACGGTGATGAATCATCTCCAATTTTAACAA
GTTTTGAATTAGTGAAAGTTCTGACCCCAAGATGTCTATGGAGAATTT
AGTTGAGAGCAAACACCACAAGCTTGCCCGGAGTTTAAAGAAGTGGACCT
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ACAAAATCTTGAAATGTGTTAATTGGGATCTACCTCAAGAGGCCAAAC
AGGCCTTGGAACCTCTGGGAAAATGGAAGCCGATGGATGTAGAGGACTC
CTTGAGCTGTTATCCTCTCATTACACCAACCCAACCTGTGAGGCGTTAT
GCTGTTGCCCGGTTGCGACAGGCCGATGATGAGGATTTGTTGATGTACC
TATTACAATTTGGTCCAGGCTCTCAAATATGAAAATTTTATGATATAAAA
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TTATAACCAGCCCCCTTCCCTCAGTCTCTTACCTCCTCCTGCATCAAA
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TCCAAAGACCCATGAGATGTACTTGAACGTAATGAGAAGATTCAGCCAA
GCATTGTTGAAGGGTGATAAGTCTGTGAGAGTTATGCGTTCTTTGCTGG
CTGCACAACAGACATTTGTAGATCGGTTGGTGCATCTAATGAAGGCAGT
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TTGCTTGAGATAATGAAAAGATGAATTTGTGAGATGTGGAACCTTATCC
CGTTGCCCTTTAGAACCCCAAGTGAATAATGAGAGGAATAATTCGGAAAC
AGCTACACTGTTTAAAGTGCCTTATGCCTGCACAGTTGTTTTTTAAG
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TACGTCAAGATCAACTTATTCTTCAAATCATTTCACTCATGGACAAGCT
GTTACGGAAAGAAAATCTGGACTTGAAATTGACACCTTATAAGGTGTTA
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TGGCTGAAGTTCTTGATACAGAGGGAAGCATTCAGAACTTTTTTTAGAAA
ATATGCACCAAGTGAGAATGGGCCAAATGGGATTAGTGCTGAGGTCATG
GACACTTACGTTAAAAGCTGTGCTGGATATTGCGTGATCACCTATATAC
TTGGAGTTGGAGACAGGCACCTGGATAACCTTTTGCTAACAAAACAGG

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CAAACCTCTCCACATAGACTTTGGATATATTTTGGGTCGGGATCCAAAG
CCTCTTCTCCACCAATGAAGCTGAATAAAGAAATGGTAGAAGGAATGG
GGGGCACACAGAGTGAGCAGTACCAAGAGTTCCGTAAACAGTGTTACAC
GGCTTTCCTCCACCTGCGAAGGTATTCTAATCTGATTTTGAACCTTGTTT
TCCTTGATGGTTGATGCAAACATTCCAGATATTGCACTTGAACCAGATA
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AGAGGCTGTGCATTACATGCAGAGTCTGATTGATGAGAGTGTCCATGCT
CTTTTTGCTGCAGTGGTGGAACAGATTCACAAGTTTGCCCAGTACTGGA
GAAAAtgagaattc

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gtcgacgccaccATGGGAAATCAGCTTGCTGGCATTGCTCCCTCCAGATCCTTTCTGTAGAGAGTTATTTTTTCAGATATTCATGACTTTGAATATGATAAAAGCCTGGGGAGTACTCGGTTTTTTAAAGTTGCTCGAGCCAAGCACCGAGAAGGCCTGGTCGTTGTGAAGGTTTTTGAATTTCAGGATCCCACATGCCTTTTAACCAGCTATAAACAAGAGCTGGAGGAACTGAAAATCAGGCTTAATTCTGCACAGAATTGTCTACCTTTCCAGAAAGCATCAGAAAAAGCATCTGAGAAAGCAGCTATGCTCTTTAGGCAGTATGTGCGAGACAATCTCTATGATCGCATCAGTACCCGTCCATTCTTGAATAACATTGAGAAGCGCTGGATTGCTTTCCAGATCCTGACAGCTGTGGACCAAGCACACAAATCTGGAGTTCGTCATGGGGACATCAAGACTGAGAATGTGATGGTCACCAGTTGGAATTGGGTTCTTCTAACTGATTTTTGCCAGTTTTTAAGCCACTTATCTTCCAGAAGACAACCCGGCAGATTTCAATTATTTCTTTGACACATCACGGAGGAGAAGTTGCTATATTGCTCCTGAACGTTTTGTTGATGGTGGGATGTTGCCACTGAGTTAGAATATATGAGAGATCCTTCAACTCCGCTTGTAAGTTAAATAGCAATCAGAGAACAAGAGGAGAGTTGAAGAGAGCAATGGACATCTTTTCAGCAGTTGTGTGATAGCTGAGCTTTTTACAGAAGGTGTACCATATTTTGATCTCTCTCAACTTTTTGGCTTATAGAAATGGACATTTTTTCCGTAACAAGTGCTAAATAAAATTGAAGATCACAGTATCAGAGAATTGGTAATCAGATGATTCACCGTGAGCCAGATAAACGTTTAGAGGCAGAAGATTACTTAAAACAGCAGCGTGCAATGCCTTTCCTGAAATATTTTTACACTTCTTTCAGCCCTACATGGCCCAGTTTGCCAAGGAAACGTTTCTTTCTGCAGATGAGCGTATTCTGGTTATACGGAAGGATTTGGGCAACATTATTCACAATCTCTGTGGACATGATCTGCCAGAAAAGCCGAAGGAGAGCCTAAGGAAATGGGCTGGTTATCTTGGTATCTGTTATAACATCCTGCCTACAGACCCTTAAATACTGTGATTCCAAACTAGCTGCTTTGGAAGTATTCTTCAATTGGCTCCAAGATTAAGTGTGAAATCCTTTTGGATCGTATTACTCCATATCTTTTGCATTTTCAGCAATGACTCTGTTCCCTAGGGTGAGGGCTGAAGCCTTGAGGACGTTGACCAAAGTTCTTGCTCTCGTCAAAGAGGTTCCCTCGTATGATATCAATATTTATCCGGAATACATTCTGCCAGGCATAGCCCCTTAGCCCAAGATGATGCTACTATCGTTAGACTAGCCTATGCTGAAAACATAGCTCTGCTGGCAGAAACAGCTCTGAGATTCCTGGAATTAGTACAGTTAAAATCTTAATATGGAAAATGACCCCAATAATGAAGAAATAGATGAGGTACACATCCAAATGGAAATTATGACACAGAGCTCCAAGCCTTACATGAAATGGTCCAGCAGAAAGTTGTTACTTTGCTAAGTGACCCTGAAAATATTGTAAAACAAACCTTGATGGAAAATGGAATAACACGGCTGTGTGTATTCTTTGGACGTCAGAAAGCCAACGATGTTTTTGTGTTGCCACATGATTACTTCTCTAAATGATAAGAATGATTGGCATCTACGTGGAGCATTTTTTTGATAGTATAGTTGGTGTGCTGCCTATGTTGGCTGGCAAAGCTCCTCAATTCTCAAGCCTCTGCTGCAACAAGGTTCTTAGTGATGCTGAGGAAATTTGTCATTGTGAAAGCTCTTTATGCCCTTACTTGTATGTGCCAGTTAGGACTGCTACAAAACCCCATGTTTACGAATTTGCCAGTGATATTGCCCCCTTCTGTGTCAATCCCAATTTATGGATACGTTATGGTGCCGTGGGATTTATCACAGTGGTAGCTCGTCAAATAAGTACAGCTGATGTCTACTGTAAACTGATGCCTTATCTTGACCCATATATTACCCAACCAATAATACAGATTGAAAGAAAACCTGTCTGCTCAGTGTTTTAAAGGAACCAGTAAGTCGTTCTATATTTGATTATGCTTTGAGGTCTAAAGATATTACTAGCTTGTTTCAGACATCTTCACATGCGTCAGAAGAAACGAAATGGTTCTCTTCCCGACTGCCCTCCGCCAGAGGA

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TCCTGCCATAGCACAGCTTCTGAAGAAGTTGCTCTCACAGGGAATGACA
GAGGAAGAGGAAGACAAACTTCTGGCACTGAAAGACTTCATGATGAAAT
CTAATAAAGCAAAGGCCAATATAGTGGACCAGAGCCATCTTCATGATAG
TAGTCAGAAAGGTGTAATTGACTTGGCAGCTTTAGGCATAACTGGGAGA
CAAGTTGATCTTGTTAAAACCAAACAAGAACCAGATGACAAACGGGCCA
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CCTGGATGGCGTCCTAAAGGGCTGTTAGTTGCCCATCTTCATGAGCATA
AATCTGCTGTGAATCGAATTAGAGTCTCTGATGAACACTCACTTTTTGC
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CTGCATTGGTACAAGCAGTGGTACCATGGCTTGTGGGACATGAGGTTTC
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TTTACTCTCTGGGCCAGCAGTGCACCACCACTTTCTGAATTACAGCCTT
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CTGTGTCCTACTACAGGAAAATAATTGAAGGCACCTGAAGTTGTCCAGGA
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CCACATTCCAGACCACACAGGGCTTCATCGTAACTGCTTCTAGAGATGG
GATTGTGAAGGTGTGGAAAaagctagcg