VCPIP CD(25-561) [GST-tagged]

Deconjugating Enzyme

Alternate Names: Valosin-containing protein p97/p47 complex-interacting protein p135, VCPIP135, KIAA1850

Cat. No.	**64-0055-050**	Quantity:	50 µg
Lot. No.	**30235**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Deconjugating enzymes (DCEs) are proteases that process ubiquitin or ubiquitin-like gene products, reverse the modification of proteins by a single ubiquitin or ubiquitin-like protein (UBL) and remodel polyubiquitin (or poly-UBL) chains on target proteins (Reyes-Turcu *et al.*, 2009). The deubiquitylating – or deubiquitinating – enzymes (DUBs) represent the largest family of DCEs and regulate ubiquitin dependent signalling pathways. The activities of the DUBs include the generation of free ubiquitin from precursor molecules, the recycling of ubiquitin following substrate degradation to maintain cellular ubiquitin homeostasis and the removal of ubiquitin or ubiquitin-like proteins (UBL) modifications through chain editing to rescue proteins from proteasomal degradation or to influence cell signalling events (Komander *et al.*, 2009). There are two main classes of DUB, cysteine proteases and metalloproteases. VCPIP is a cysteine protease and is a member of the OTU superfamily of proteins. Cloning of the human gene was first described by Nagase *et al.* (2001). OTU enzymes play important roles as negative-feedback regulators in NF-κB signalling, interferon signalling and in p97 (cdc48)-mediated processes although the cellular functions of most OTU enzymes remain to be discovered. Ovarian tumour family DUBs contain a papain-like catalytic core of ~180 amino acids. In addition to their catalytic domain, many OTU members have additional ubiquitin-binding domains (UBDs). At least 20 different UBD families have

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: ~87 kDa

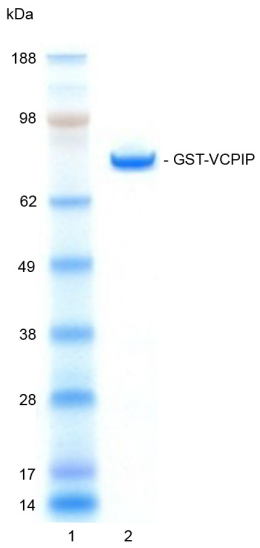
Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

Quality Assurance

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



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-VCPIP was validated by determining the increase in fluorescence measured as a result of the enzyme catalysed cleavage of the fluorogenic substrate Ubiquitin-Rhodamine110-Glycine generating Ubiquitin and Rhodamine110-Glycine. Incubation of the substrate in the presence or absence of GST-VCPIP was compared confirming the deubiquitylating activity of GST-VCPIP.

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Lot-specific COA version tracker: v1.0.0

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Background

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been described, and knowledge of linkage-specific UBDs have provided the means to understand the roles of different ubiquitin linkages in cells (Licchesi *et al.*, 2012). VCPIP was originally identified as a p97/p47 complex-interacting protein, which binds to the p97/p47/SNARE complex and assists its dissociation via p97-catalysed ATP hydrolysis (Uchiyama *et al.*, 2002). Wang *et al.* (2004) subsequently reported that VCPIP possesses deubiquitylating activity, which is essential for p97/p47-mediated Golgi membrane fusion. The deubiquitylating activity of VCPIP is, however, unnecessary for p97/p37-mediated Golgi membrane fusion, although VCPIP itself is essential in this pathway. VCPIP therefore seems to work in two distinct ways, one via its deubiquitylating activity and the other in a ubiquitin-independent manner, which may be as a p97/p47 (or p37) complex-interacting protein (Uchiyama *et al.*, 2006). VCPIP is a member of the A20-like subfamily; other members include A20, Cezanne, Cezanne2 and TRABID. VCPIP - and OTUD2 - efficiently cleave K11-linked chains and interact with p97, suggesting that p97 may act on substrates containing atypical linkage types, although the role of DUBs in p97 function is not well understood (Mevissen *et al.*, 2013).

References:

Komander D, Clague MJ and Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* **10**, 550-563.

Licchesi JD, Mieszczanek J, Mevissen TE, Rutherford TJ, Akutsu M, Virdee S, et al. (2012) An ankyrin-repeat ubiquitin-binding domain determines TRABID's specificity for atypical ubiquitin chains. *Nature Structural & Molecular Biology* **19**, 62-71.

Mevissen TE, Hospenthal MK, Geurink PP, Elliott PR, Akutsu M, Arnaudo N, Ekkebus R, Kulathu Y, Wauer T, El Oualid F, Freund SM, Ovaa H, Komander D (2013) OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. *Cell* **154**, 169-84.

Nagase T, Nakayama M, Nakajima D, Kikuno R, Ohara O (2001) Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res* **8**, 85-95.

Reyes-Turcu FE, Ventii KH and Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. Ann Rev Biochem **78**, 363-397.

Uchiyama K, Jokitalo E, Kano F, Murata M, Zhang X, Canas B, Newman R, Rabouille C, Pappin D, Freemont P, Kondo H (2002) VCIP135, a novel essential factor for p97/p47-mediated membrane fusion, is required for Golgi and ER assembly *in vivo*. *J Cell Biol* **159**, 855–866.

Uchiyama K, Totsukawa G, Puhka M, Kaneko Y, Jokitalo E, Dreveny I, Beuron F, Zhang X, Freemont P, Kondo H (2006) p37 is a p97 adaptor required for Golgi and ER biogenesis in interphase and at the end of mitosis. *Dev Cell* **11**, 803–816.

Wang Y, Satoh A, Warren G, Meyer HH (2004) VCIP135 acts as a deubiquitinating enzyme during p97-p47-mediated reassembly of mitotic Golgi fragments. *J Cell Biol* **164**, 973–978.

Physical Characteristics

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Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHN
MLGGCPKERAEISMLEGAVLDIRYGVS
RIAYSKDFETLKVDFLSKLPEMLKMFE
DRLCHKTYLNGDHVTHPDFMLYDALDV
VLYMDPMCLDAFPKLVCFKKRIEAIPQ
IDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSDLEVLFQGPLGSPEFM***S***SLASAAAS
GGLLKRRDRRILSGSCPDPKCQARLFF
PASGSVSIECTECGQRHEQQQLLGVEEVTDP
DVVLHNLLRNALLGVTGAPKKNTELVKVMGL
SNYHCKLLSPILARYGMDKQTGRAKLLRDM
NQGELFDCALLGDRAFLIEPEHVNTVGYG
KDRSGSLLYLHDTLEDIKRANKSQECLIPVH
VDGDGHCLVHAVSRALVGRELFWHALREN
LKQHFQQHLARYQALFHDFIDAAEWEDI
INECDPLFVPPEGVPLGLRNIHIFGLANV
LHRPIILLDSLSGMRSSGDYSATFLPGLI
PAEKCTGKDGHLNKPICIAWSSSGRNHYI
PLVGIKGAALPKLPMNLLPKAWGVPQD
LIKKYIKLEEDGGCVIGGDRSLQDKYLLR
LVAAMEEVFMDKHGIHPSLVADVHQYFYR
RTGVIGVQPEEVTAAAKKAVMDNRLHKCLL
CGALSELHVPPEWLAPGGKLYNLAKSTHGQL
RTDKNYSFPLNNLVCSYDSVKDVLVPDYGM
SNLTACNWCHGTSVRKVRGDGSIVYLDGD

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
VCPIP (regular text): Start ***bold italics*** (amino acid residues 25-561)
Accession number: NP_079330

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