TRABID CD(245-697) [6His-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: TRAF binding protein domain, Zinc finger RAN binding domain containing 1, ZRANB1

Cat. No.	**64-0049-050**	Quantity:	50 µg
Lot. No.	**30166**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

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. TRABID is a cysteine protease and is a member of the OTU (ovarian tumour) superfamily of proteins (Balakirev *et al.,* 2003). Cloning of the human gene was first described by Evans *et al.* (1992). TRABID was recently reported to specifically and positively regulate the Wnt signalling pathway. TRABID is composed of a tumour necrosis factor receptor associated factor (TRAF) binding domain in the C-terminus and three Zinc-finger (ZnF) motifs at the N-terminus. TRABID preferentially binds to K63-linked polyubiquitin chains but not K48-linked polyubiquitin chains and specifically cleaves

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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: ~54.8 kDa

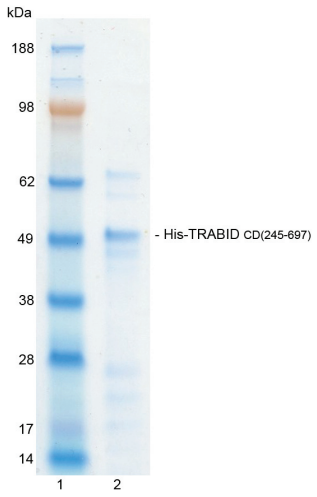
Purity: >81% by InstantBlue™ SDS-PAGE

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 His-TRABID CD(245-697)



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of His-TRABID CD(245-697) was validated by the monitoring of mono-ubiquitin generation as a result of the enzyme catalysed cleavage of K63-linked di-ubiquitin. Incubation of the substrate in the presence or absence of His-TRABID CD(245-697) was compared confirming the deubiquitylating activity of His-TRABID CD(245-697).

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Lot-specific COA version tracker: v1.0.0

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K63 chains. K63-linked ubiquitylation is known to fulfil diverse proteasome-independent roles, including DNA repair, endocytosis and NFκB signalling (Shi *et al.,* 2012). Proteins that mediate specific assembly and disassembly of atypical K6, K27, K29 and K33 linkages are mainly unknown. Recent work on these atypical di-ubiquitins has shown that TRABID specifically hydrolyses both K29 and K33-linked di-ubiquitin (Licchesi *et al.,* 2012) and in fact cleaves the K29 linkage with 40-fold more efficiency than the K63 linkage (Virdee *et al.,* 2010)

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.

Nakamura T, Hillova J, Mariage-Samson R, Onno M, Huebner K, Cannizzaro LA, *et al.* (1992) A novel transcriptional unit of the tre oncogene widely expressed in human cancer cells. *Oncogene* **7**, 733-741

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

Shi T, Bao J, Wang NX, Zheng J and Wu D (2012) Identification Of Small Molecule TRABID Deubiquitinase Inhibitors By Computation-Based Virtual Screen. *BMC Chem Biol* **12**, 4.

Virdee S, Ye Y, Nguyen DP, Komander D and Chin JW (2010) Engineered diubiquitin synthesis reveals Lys29-isopeptide specificity of an OTU deubiquitinase. *Nature Chemical Biology* **6**, 750-757.

Physical Characteristics

Continued from page 1

Protein Sequence:

MGSSHHHHHHSSGLEVLFQGPRS**L**EVDFK
KLKQIKNRMKKTDWLFLNACVGVVEGD
LAAIEAYKSSGGDIARQLTADEVRLLNRP
SAFDVGYTLVHLAIRFQRQDMLAILLTE
VSQQAAKCIPAMVCPELTEQIRRE
IAASLHQRKGDFACYFLTDLVTFTLPAD
IEDLPPTVQEKLFDEVLDRDVQKELEEESPI
INWSLELATRLDSRLYALWNRTAGDCLLDS
VLQATWGIYDKDSVLRKALHDSLHDCSHW
FYTRWKDWESWYSQSFGLHFSLREEQWQED
WAFILSLASQPGASLEQTHIFVLAHILRRPI
IVYGVKYYKSFRGETLGYTRFQGVYLPLL
WEQSFCWKSPIALGYTRGHFSALVA
MENDGYGNRGAGANLNTDDDVTITFLPLVD
SERKLLHVHFLSAQELGNEEQQEKLLREWLDC
CVTEGGVLVAMQKSSRRRNHPLVTQMVEKWLD
RYRQIRPCTSLS

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
TRABID CD(246-697) (regular text): Start ***bold italics*** (amino acid residues 245-697)
Accession number: NP_060050

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