USP30 CD(57-517) [untagged]

Deconjugating Enzyme

Alternate Names: Ubiquitin carboxyl-terminal hydrolase 30, Ubiquitin thioesterase 30, Ubiquitin-specific-processing protease 30

Cat. No.	**64-0057-050**	Quantity:	50 µg
Lot. No.	**30327**	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. Ubiquitin specific protease 30 (USP30) is a member of the cysteine protease enzyme family and cloning of the gene was first described by Quesada *et al.* (2004). USP30 can be found anchored to the mitochondrial outer-membrane (MOM), contributing to the regulatory mechanism for mitochondrial dynamics in mammalian cells. A loss of USP30 activity has been shown to disturb the maintenance of mitochondrial morphology and lead to mitochondrial elongation (Nakamura and Hirose, 2008). Loss-of-function mutations in PINK1 kinase and the ubiquitin E3 ligase Parkin, are a significant cause of early on-

Continued on page 2

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: ~53 kDa

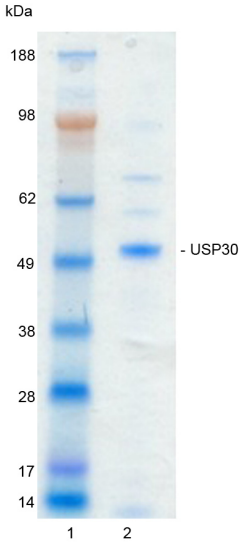
Purity: >67% by InstantBlue™ SDS-PAGE

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 USP30



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of USP30 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 USP30 was compared confirming the deubiquitylating activity of USP30.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
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

USP30 CD(57-517) [untagged]

Deconjugating Enzyme

Alternate Names: Ubiquitin carboxyl-terminal hydrolase 30, Ubiquitin thioesterase 30, Ubiquitin-specific-processing protease 30

Cat. No.	**64-0057-050**	Quantity:	50 µg
Lot. No.	**30327**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

Background

Continued from page 1

set Parkinson's disease. PINK1 activates Parkin to induce selective autophagy of damaged mitochondria. After recruitment, Parkin mediates the engulfment of mitochondria by autophagosomes and the selective elimination of impaired mitochondria. Parkin promotes autophagy of damaged mitochondria and implicates a failure to eliminate dysfunctional mitochondria in the pathogenesis of Parkinson's disease (Kane *et al.*, 2014; Narendra *et al.*, 2008). A small molecule inhibitor of USP30 has recently been identified and reported. The diterpenoid derivative is a natural molecule named 15-oxospiramilactone (S3) that induces mitochondrial fusion to restore the mitochondrial network and oxidative respiration in cells that are deficient in mitochondrial fusion genes mitofusin 1 (Mfn1) or mitofusin 2 (Mfn2). The inhibition of USP30 by S3 leads to an increase of Mfn1/2 activity promoting mitochondrial fusion (Yue *et al.*, 2014).

References:

Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, *et al.* (2014) PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol* **205**, 143-153.

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.

Nakamura N and Hirose S (2008) Regulation of mitochondrial morphology by USP30, a deubiquitinating enzyme present in the mitochondrial outer membrane. *Mol Biol Cell* **19**, 1903-1911.

Narendra D, Tanaka A, Suen DF and Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol* **183**, 795-803.

Quesada V, Diaz-Perales A, Gutierrez-Fernandez A, Garabaya C, Cal S and Lopez-Otin C (2004) Cloning and enzymatic analysis of 22 novel human ubiquitin-specific proteases. *Biochem Biophys Res Commun* **314**, 54-62.

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

Yue W, Chen Z, Liu H, Yan C, Chen M, Feng D, *et al.* (2014) A small natural molecule promotes mitochondrial fusion through inhibition of the deubiquitinase USP30. Cell Res **24**, 482-496.

Physical Characteristics

Continued from page 1

Protein Sequence:

P L G S ***T*** E R K K R R K G L V P G L V N L
G N T C F M N S L L Q G L S A C P A F I R
W L E E F T S Q Y S R D Q K E P P S H Q Y L S L T L L
H L L K A L S C Q E V T D D E V L D A S C L L D V L
R M Y R W Q I S S F E E Q D A H E L F H V I T S S L E
D E R D R Q P R V T H L F D V H S L E Q Q S E I T P K Q
I T C R T R G S P H P T S N H W K S Q H P F H G R L T S N M
V C K H C E H Q S P V R F D T F D S L S L S I P A A T W G H
P L T L D H C L H H F I S S E S V R D V V C D N C T K I E A K
G T L N G E K V E H Q R T T F V K Q L K L G K L P Q C L C I
H L Q R L S W S S H G T P L K R H E H V Q F N E F L M M D I
Y K Y H L L G H K P S Q H N P K L N K N P G P T L E L Q D G P
G A P T P V L N Q P G A P K T Q I F M N G A C S P S L L P T L
S A P M P F P L P V V P D Y S S S T Y L F R L M A V V V H
H G D M H S G H F V T Y R R S P P S A R N P L S T S N Q W L
W V S D D T V R K A S L Q E V L S S S A Y L L F Y E R V
L S R M Q H Q S Q E C K S E E

The residues underlined remain after cleavage and removal of the purification tag.
USP30 (regular text): Start ***bold italics*** (amino acid residues 57-517)
Accession number: NP_116052

UBIQUIGENT™
www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
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