JOSD1 [6His-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Name: KIAA0063

Cat. No.	**64-0031-050**	Quantity:	50 µg
Lot. No.	**30074**	Storage:	-70˚C

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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. JOSD1 is a cysteine protease and a member of the Machado-Joseph Domain (MJD) enzyme family. Cloning of the human gene was first described by Nomura *et al.* (1994). The Josephin domain is a conserved cysteine protease domain found in four human deubiquitylating enzymes: ataxin-3, the ataxin-3-like protein (Ataxin-3L), Josephin-1 (JOSD1), and Josephin-2 (JOSD2). Two of the human Josephin proteins, ataxin-3 and ataxin-3L, each contain a single Josephin domain at their N-terminus plus a flexible C-terminal domain of compara-

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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: ~26 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

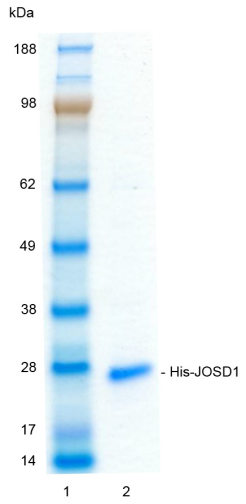
Protein Sequence:

MGSSHHHHHHSSGLEVLFQGPGS***M***SCVPWK
GDKAKSESLELPQAAPPQIYHEKQRRELCAL
HALNNVFQDSNAFTRDTLQEIFQRLSPNTMVT
PHKKSMLGNGNYDVNVIMAALQTKGYEAVWWD
KRRDVGVIALTNVMGFIMNLPSSLCWGPLKL
PLKRQHWICVREVGGAYYNLDSKLKMPEWIG
GESELRKFLKHHLRGKNCELLLVVPEE
VEAHQSWRTDV

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
JOSD1 (regular text): Start ***bold italics*** (amino acid residues 1-202)
Accession number: NP_055691

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-JOSD1



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of His-JOSD1 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 His-JOSD1 was compared confirming the deubiquitylating activity of His-JOSD1.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

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ble length. JOSD1 and JOSD2, on the other hand, are each composed solely of a single Josephin domain (Weeks *et al.*, 2011). JOSD1 and JOSD2 have been shown to possess DUB activity although details of their substrate specificity are still largely unknown (Tzvetkov and Breuer, 2007).

References:

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

Nomura N, Nagase T, Miyajima N, Sazuka T, Tanaka A, Sato S, Seki N, Kawarabayasi Y, Ishikawa K, Tabata S (1994) Prediction of the coding sequences of unidentified human genes. II. The coding sequences of 40 new genes (KIAA0041-KIAA0080) deduced by analysis of cDNA clones from human cell line KG-1. *DNA Res* **1**, 223-229.

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

Tzvetkov N , Breuer P (2007) Josephin domain-containing proteins from a variety of species are active de-ubiquitination enzymes. *Biol Chem* **388**, 973-978.

Weeks SD, Grasty KC, Hernandez-Cuebas L, Loll PJ (2011) Crystal structure of a Josephin-ubiquitin complex: evolutionary restraints on ataxin-3 deubiquitinating activity. *J Biol Chem* **286**, 4555-4565.

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Lot-specific COA version tracker: v1.0.0