## **JOSD2** [6His-tagged] Deconjugating enzyme: Deubiquitylase

Alternate Name: Hypothetical transmembrane protein SBBI54

Cat. No. Lot. No.	64-0032-050 30075	

Quantity: 50 µg 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 ubiguitin from precursor molecules, the recycling of ubiquitin following substrate degradation to maintain cellular ubiquitin homeostasis and the removal of ubiguitin 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. JOSD2 is a cysteine protease and a member of the Machado-Joseph Domain (MJD) enzyme family. Cloning of the human gene was first described by Gerhard et al. (2004). The Josephin domain is a conserved cysteine protease domain found in four human deubiguitylating 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: ~23 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

### **Protein Sequence:**

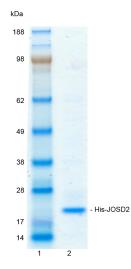
MGSSHHHHHHSSGLEVLFQGPGSMSQAP GAQPSPPTVYHERQRLELCAVHALN NVLQQQLFSQEAADEICKRLAPDSRLN PHRSLLGTGNYDVNVIMAALQGLGLAAVW WDRRRPLSQLALPQVLGLILNLPSPVS LGLLSLPLRRHWVALRQVDGVYYNLDSKLRA PEALGDEDGVRAFLAAALAQGLCEVLLVVT KEVEEKGSWLRTD

Tag (**bold text**): N-terminal His Protease cleavage site: PreScission <sup>™</sup> (<u>LEVLFQ▼GP</u>) JOSD2 (regular text): Start **bold italics** (amino acid residues 1-188) Accession number: NP\_612207

## **Quality Assurance**

#### **Purity:**

4-12% gradient SDS-PAGE InstantBlue™ staining Lane 1: MW markers Lane 2: 1 μg His-JOSD2



Protein Identification:

Confirmed by mass spectrometry.

#### Deubiquitylase Enzyme Assay:

The activity of His-JOSD2 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-JOSD2 was compared confirming the deubiquitylating activity of His-JOSD2.



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Lot-specific COA version tracker: v1.0.0

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## Background

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#### Continued from page 1

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:

Gerhard DS, Wagner L, Feingold EA, Shenmen CM, Grouse LH, Schuler G, *et al.* (2004) The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC). *Genome Res* **14**, 2121-2127.

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

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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