WWP1 [untagged]

E3 Ligase

Alternate Names: AIP5; Atropin 1 interacting protein 5; hSDRP1; Nedd 4 like ubiquitin protein ligase; TGIF interacting ubiquitin ligase 1; Tiul1; WW domain containing E3 ubiquitin protein ligase 1

Cat. No.	**63-0034-025**	Quantity:	25 µg
Lot. No.	**30033**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). WW domain containing Protein (WWP1) is a member of the E3 protein ligase family and cloning of the human gene was first described by Pirozzi *et al.* (1997). WWP1 belongs to the NEDD4 protein family and contains 4 WW domains (Flasza *et al.*, 2002; Pirozzi *et al.*, 1997). The intrinsic E3 ligase activity of WWP1 is conferred through a HECT domain at the C-terminus of the protein (Pirozzi *et al.*, 1997). WWP1 has been shown to interact with Smad7 in human epithelial cell lines to cause the ubiquitylation and degradation of Transforming Growth Factor Beta Receptor-1 (TGFβR-1) (Seo *et al.*, 2004). Treatment of human embryonic kidney cells with TGFβ also leads to the ubiquitylation and degradation of SMAD2 through the interaction of SMAD2/SMAD3, and the nuclear co-repressor Transforming Growth Factor Beta-Induced Factor (TGIF) with WWP1 (Seo *et al.*, 2004).

References:

Flasza M, Gorman P, Roylance R, Canfield AE, Baron M (2002) Alternative splicing determines the domain structure of WWP1, a Nedd4 family protein. *Biochem Biophys Res Commun* **290**, 431-7.

Pirozzi G, McConnell SJ, Uveges AJ, Carter JM, Sparks AB, Kay BK, Fowlkes DM (1997) Identification of novel human WW domain-containing proteins by cloning of ligand targets. *J Biol Chem* **272**, 14611-6.

Seo SR, Lallemand F, Ferrand N, Pessah M, L'Hoste S, Camonis J, Atfi A (2004) The novel E3 ubiquitin ligase Tiul1 associates with TGIF to target Smad2 for degradation. *EMBO J* **23**, 3780-92.

Physical Characteristics

Species: human

Source: *E. coli* expression

Quantity: 25 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: ~105 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

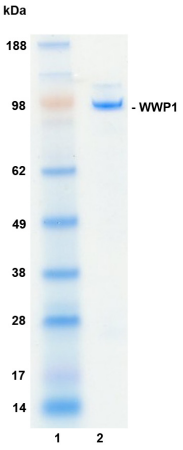
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

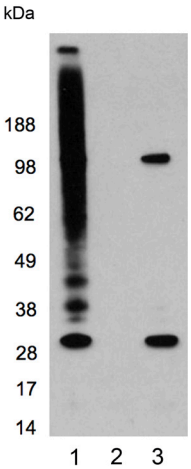
Quality Assurance

Protein Identification:
Confirmed by mass spectrometry.

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg WWP1



E3 ligase assay:
The ubiquitin conjugating activity of WWP1 was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2L3 (UbcH7) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of WWP1 for 60 minutes at 37˚C in the presence of ubiquitin, His-UBE1, His-UBE2L3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or WWP1 (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-ubiquitin conjugate antibody and these were observed only in the presence of both ATP and WWP1 (with the exception of one species of approximately 100 MW observable in lane 3).



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

Lot-specific COA version tracker: v1.0.1

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E3 Ligase

Alternate Names: AIP5; Atropin 1 interacting protein 5; hSDRP1; Nedd 4 like ubiquitin protein ligase; TGIF interacting ubiquitin ligase 1; TiuI1; WW domain containing E3 ubiquitin protein ligase 1

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Physical Characteristics

Protein Sequence:

GPLGS***I***ATASPRSDTSNNHSGRLQLQVT
VSSAKLKRKKNWFGTAIYTEVVVDGEIT
KTAKSSSSSNPKWDEQLTVNVTPQT
TLEFQVWSHRTLKADALLGKATIDLKQAL
LIHNRKLERVKEQLKLSLENKNGIAQT
GELTVVLDGLVIEQENITNCSSSP
TIEIQENGDALHENGEPSARTTAR
LAVEGTNGIDNHVPTSTLVQNSCCSYV
VNGDNTPSSPSQVAARPKNTPAPK
PLASEPADDTVNGESSSFAPTDNAS
VTGTPVVSEENALSPNCTSTTVEDP
PVQEILTSSENNECIPSTSAELESEAR
SILEPDTSNSRSSSAFEAAKSRQPDGCM
DPVRQQSGNANTETLPSGWEQRKDPHGR
TYYVDHNTRTTTWERPQPLPPGWERRVD
DRRRVYYVDHNTRTTTWQRPTMESVRN
FEQWQSQRNQLQGAMQQFNQRYLYSASM
LAAENDPYGPLPPGWEKRVDSTDRVY
FVNHNTKTTQWEDPRTQGLQNEEPLPEG
WEIRYTREGVRYFVDHNTRTTTFKD
PRNGKSSVTKGGPQIAYERGFRWKLAH
FRYLCQSNALPSHVKINVSRQTLFEDS
FQQIMALKPYDLRRRLYVIFRGEEGLDYG
GLAREWFFLLSHEVLNPMYCLFEYAG
KNNYCLQINPASTINPDHLSYFCFIGR
FIAMALFHGKFIDTGFSLPFYKRMLSK
KLTIKDLESIDTEFYNSLIWIRDNNIEEC
GLEMYFSVDMEILGKVTSHDLKLGGSNIL
VTEENKDEYIGLMTEWRFSRGVQEQTKA
FLDGFNEVVPLQWLQYFDEKELEVML
CGMQEVDLADWQRNTVYRHYTRNSKQI
IWFWQFVKETDNEVRMRLLQFVTGTCRL
PLGGFAELMGSNGPQKFCIEKVGKDTWL
PRSHTCFNRLDLPPYKSYEQLKEKLLFA
IEETEGFGQE

The residues underlined remain after cleavage and removal of the purification tag.
WWP1 (regular text): Start ***bold italics*** (amino acid residues 2-922
Accession number: NP_008944

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Lot-specific COA version tracker: v1.0.1