

# WWP1 [GST-tagged]

## 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.** 63-0033-025

**Lot. No.** 30029

**Quantity:** 25 µg

**Storage:** -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

## 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:** ~132 kDa

**Purity:** >95% by InstantBlue™ SDS-PAGE

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

**Protein Sequence:** Please see page 2

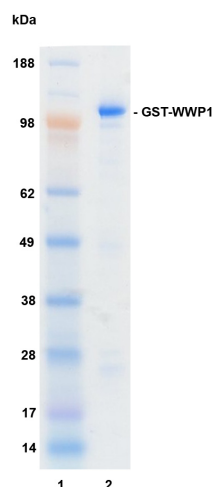
## Quality Assurance

### Protein Identification:

Confirmed by mass spectrometry.

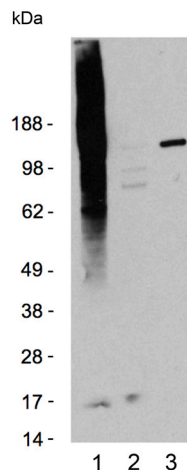
### Purity:

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



### E3 ligase assay:

The ubiquitin conjugating activity of GST-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 GST-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 GST-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 GST-WWP1 (with the exception of one species of approximately 140 MW observable in lane 3).



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Lot-specific COA version tracker: v1.0.1

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## Physical Characteristics

### Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY  
EEHLYERDEGDKWRNKKFELGLEFPN  
LPYYIDGDVTKLTQSMAIRYIADKHN  
MLGGCPKERAESMLEGAVLDIRYGV  
RIAYSKDFETLKVDFLSKLPPEMLKMF  
DRLCHKTYLNGDHVTHPDFMLYDALDV  
VLYMDPMCLDAFPKLVCFKKRIEAIPO  
IDKYLKSSKYIAWPLQGWQATFGGGDHP  
PKSDLEVLFGGPLGS IATASPRSDTSN  
NHSGRLLQLQVTVSSAKLKRKKNWFGTAI  
YTEVVVDGEITKTAKSSSSSNPKWDEQLT  
VNVTPQTTLEFQVWSHRTLKADALLG  
KATIDLKQALLIHNKLERVKEQLKLSLEN  
KNGIAQTGELTVVLDGLVIEQENIT  
NCSSSPTIEIQENGDALHENGEPART  
TARLAVEGTNGIDNHVPTSTLVQN  
SCCSYVNGDNTSPSSPSQVAARPKNT  
PAPKPLASEPADDTVNGESSFAPTD  
NASVTGTPVVSEENALSPNCTSTTVEDP  
PVQEILTSENNECIPSTSAELESEAR  
SILEPDTNSRSSFEEAAKSRQPDGCM  
DPVRQQSGNANTETLPSGWEQRKDPHGR  
TYVVDHNTRTTTWERPQPLPPGWERRVD  
DRRRVYYVDHNTRTTTWQRPTMESVRN  
FEQWQSQRNQLQGAMQQFNQRYLYSASM  
LAAENDPYGPLPPGWEKRVDS TDRVY  
FVNHNTKTTQWEDPRTQGLQNEEPLPEG  
WEIRYTRREGVRYFVDHNTRTTTFKD  
PRNGKSSVTKGGPQIAYERGFRWKL AH  
FRYLCQSNALPSHVKINVSRTLFEDS  
FQQIMALKPYDLRRRLYVIFRGEGLDYG  
GLAREWFFLLSHEVLNPMYCLFEYAG  
KNNYCLQINPASTINPDHLSYFCFIGR  
FIAMALFHGKFIDTGFSLPFYKRMLSK  
KLTIKDLESIDTEFYNSLIWIRDNNIEEC  
GLEMYFSVDMEILGKVTSHDLKLGGSNIL  
VTEENKDEYIGLMTEWRFSRGVQEQTKA  
FLDGFNEVVPLQWLQYFDEKELEVML  
CGMQEVDLADWQRNTVYRHYTRNSKQI  
IWFQFVKETDNEVRMRLQLFVTGTCL  
PLGGFAELMGSNGPQKFCIEKVGKDTWL  
PRSHTCFNRLDLPPYKSYEQLEKLLFA  
IEETEGFGQE

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (LEVLQVGP)

WWP1 (regular text): Start **bold italics** (amino acid residues 2-922)

Accession number: NP\_008944



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