

HUWE1 [GST-tagged]

E3 Ligase

Alternate Names: HECT domain protein LASU1, UREB1

Cat. No. 63-0042-025

Lot. No. 30227

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). Hect, Uba, and Wwe Domains containing protein 1 (HUWE1) is a member of the Homologous to the E6AP Carboxyl Terminus (HECT) E3 protein ligase family and cloning of the human gene was first described by (Liu et al., 2005). HUWE1 ubiquitylates the Histone proteins, H1, H2A, H2B, H3 and H4 (Liu et al., 2005). PCR analysis of X chromosome-specific array comparative genomic hybridization has identified over expression of HUWE1 in the blood of individuals affected by non-syndromic X-linked mental retardation (Froyen et al., 2008). HUWE1 catalyses K48 linked poly-ubiquitylation and proteasomal degradation of the transcription factor Miz1 (Myc-Interacting Zinc finger protein 1) (Yang et al., 2010). HUWE1 has also been shown to ubiquitylate Mcl1, p53, MyoD and cMyc (Zhong et al., 2005; Chen et al., 2005; Noy et al., 2012; Inoue et al., 2013). HUWE1 null mice display increased number and severity of skin tumors which can be reversed by concomitant genetic knockout of c-Myc (Inoue et al., 2013).

References:

Chen D, Kon N, Li M, Zhang W, Qin J, Gu W (2005) ARF-BP1/Mule is a critical mediator of the ARF tumor suppressor. *Cell* 121, 1071–1083.

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Physical Characteristics

Species: human

Source: *E. coli*

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: ~97 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

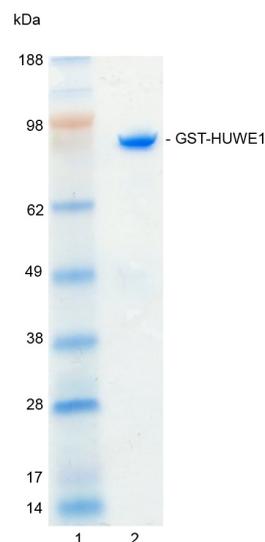
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg GST-HUWE1



Protein Identification:

Confirmed by mass spectrometry.



E3 ligase assay: The ubiquitin conjugating activity of GST-HUWE1 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-UBE2D3 (UbcH5c) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-HUWE1 for 60 minutes at 30°C in the presence of ubiquitin, His-UBE1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-HUWE1 (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-HUWE1.



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

Froyen G, Corbett M, Vandewalle J, Jarvela I, Lawrence O, Meldrum C, Bauters M, Govaerts K, Vandeleur L, Van Esch H, Chelly J, Sanlaville D, *et al.* (2008) Submicroscopic duplications of the hydroxysteroid dehydrogenase HSD17B10 and the E3 ubiquitin ligase HUWE1 are associated with mental retardation. *Am J Hum Genet* 82, 432-443.

Liu Z, Oughtred R, Wing SS. (2005) Characterization of E3-Histone, a novel testis ubiquitin protein ligase which ubiquitinates histones. *Molec Cell Biol* 25, 2819-2831.

Inoue S, Hao Z, Elia AJ, Cescon D, Zhou L, *et al.* (2013) Mule/Huwe1/Arf-BP1 suppresses Ras-driven tumorigenesis by preventing c-Myc/Miz1-mediated down-regulation of p21 and p15. *Genes Dev* 27, 1101-14.

Noy T, Suad O, Taglicht D, Ciechanover A. (2012) HUWE1 ubiquitinates MyoD and targets it for proteasomal degradation. *Biochem Biophys Res Commun* 418, 408-13.

Yang Y, Do H, Tian X, Zhang C, Liu X, Dada LA, Sznajder JI, Liu J. (2010) E3 ubiquitin ligase Mule ubiquitinates Miz1 and is required for TNFalpha-induced JNK activation. *PNAS* 27, 13444-9.

Zhong Q, Gao W, Du F, Wang X. (2005) Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. *Cell* 121, 1085-1095.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSR IAY
SKDFETLKVDFLSKLPPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAIPOIDKYLKSSKY
IAWPLQGWQATFGGGDHPPKSDENLYFQGG
SIQAAVRQLEAEADAI IQMVREGQRRAR
RQQQAATSESSQSEASVRREESPMVDVQSP
SAQDTQSIASDGTPOGEKEKEERPEL
PLLSEQLSLDELWDMLGECLEKELEESH
DQHAVLVLPVEAFFLVHATERESKP
PVRDTRESQLAHIKDEPPPLSPAPLT
PATPSSLDPPFSREPSSMHISSSLPPDTQK
FLRFAETHRTVLNQLRQSTTHLADGPFVAV
LVDYIRVLDFDVKKRYFRQELERLDEGL
RKEDMAHVRRDHVFEEDSYRELHRKSPEEM
KNRLYIVFEGEEGQDAGLLREWYMIIS
REMFNPMYALFRTPGDRVTTYINPSSHCHN
PNHLSYFKFVGRIVAKAVYDNRLLECYFTR
FYKHILGKSVRYTDMESDYHFYQGLVYL
LENDVSTLGYDLTFSTEVQEFVCEVRDLK
PNGANILVTEENKKEYVHLVCQMRMT
GAIRKQLAAFLEGFYEIIPKRLISIFT
EQELELLISGLPTIDIDDLKSNTEYHKY
QNSIQIQWFWRALRSFDQADRAKFLQFVT
GTSKVPLQGFAALEGMNGIQKFIHRDDRST
DRLPSAHTCFNQLDLPAYESFEKLRHMLL
LAIQECSEGFGLA

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

Protease cleavage site: TEV™ (**ENLYF▼QG**)

HUWE1 (regular text): Start **bold italics** (amino acid residues 3760-4374)

Accession number: NP_113584.3



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