

UBA6 [6His-tagged]

E1 - Ubiquitin Activating Enzyme

Alternate Names: E1-L2, UBE1L2

Cat. No. 61-0002-010
Lot. No. 1367

Quantity: 10 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 regulated and targeted proteosomal 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). UBA6 is a member of the E1 activating enzyme family and the human gene was first described by Jin *et al.* (2007). UBA6 shares 42% homology with UBE1 and contains all the structural elements required for E1 enzyme activity (Groettrup *et al.*, 2008). UBA6 interacts with a number of E2 and E3 enzymes and has been shown to be involved with p53 ubiquitylation *in vitro* (Groettrup *et al.*, 2008; Pelzer *et al.*, 2007). UBA6 activates ubiquitin and the ubiquitin-like protein human leukocyte antigen F-Associated Transcript 10 (FAT10), both of which may serve as a signal for proteasomal degradation. FAT10, is encoded by the major histocompatibility (MHC) class I locus, and its expression is induced by tumor necrosis factor alpha (TNF α) and interferon-gamma (IFN γ). FAT10 expression is significantly upregulated in hepatocellular carcinoma as well as in gastrointestinal and gynecological cancers (Lee *et al.*, 2003), however its precise biochemical and cellular functions have yet to be determined. Although FAT10 forms covalent conjugates with cellular proteins through its C-terminal diglycine motif (Raasi *et al.*, 2001), substrates remain to be identified. Knockdown of UBA6 results in decreased FAT10 conjugation, indicating that UBA6 is required to activate FAT10 and facilitate its conjugation (Chiu *et al.*, 2007).

References:

Chiu YH, Sun Q, Chen ZJ (2007) E1-L2 activates both ubiquitin and FAT10. *Mol Cell* **27**, 1014-23.

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Physical Characteristics

Species: human

Source: Sf21 insect cell-baculovirus expression

Quantity: 10 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: ~123 kDa

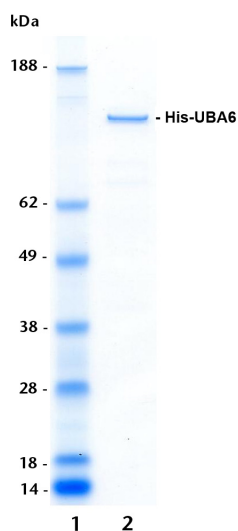
Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg His-UBA6



Protein Identification:

Confirmed by mass spectrometry.

E1-Ubiquitin Thioester Loading Assay:

The activity of His-UBA6 was validated by loading ubiquitin onto the active cysteine of His-UBA6. Incubation of the His-UBA6 enzyme in the presence of ubiquitin and ATP at 30°C was compared at two time points, T₀ and T₁₀ minutes. Sensitivity of the ubiquitin/His-UBA6 thioester bond to the reducing agent DTT was confirmed.



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

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Groettrup M, Pelzer C, Schmidtke G, Hofmann K (2008) Activating the ubiquitin family: UBA6 challenges the field. *Trends Biochem Sci* **33**, 230-7.

Jin J, Li X, Gygi SP, Harper JW (2007) Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. *Nature* **447**, 1135-8.

Lee CG, Ren J, Cheong IS, Ban KH, Ooi LL, Yong Tan S, Kan A, Nuchprayoon I, Jin R, Lee KH, Choti M, Lee LA (2003) Expression of the FAT10 gene is highly upregulated in hepatocellular carcinoma and other gastrointestinal and gynecological cancers. *Oncogene* **22**, 2592-603.

Pelzer C, Kassner I, Matentzoglou K, Singh RK, Wollscheid HP, Scheffner M, Schmidtke G, Groettrup M (2007) UBE1L2, a novel E1 enzyme specific for ubiquitin. *J Biol Chem* **282**, 23010-4.

Raasi S, Schmidtke G, Groettrup M (2001) The ubiquitin-like protein FAT10 forms covalent conjugates and induces apoptosis. *J Biol Chem* **276**, 35334-43.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSYYHHHHHDYDIPTTENLYFQGAMGS
GIQRPTSTSSLVAAA**MEG**SEPVAAHQGEEASC
SSWGTGSTNKNLPIMSTASVEIDDALYSRQRYV
LGDTAMQKMAKSHVFLSGMGGLGLEIAKN
LVLGAIKAVTIHDTEKQAWDLGTFNFFLSED
DVVNKRNRAEAVLKHIAELNPYVHVTSVSPF
NETDLSFLDKYQCVLTEMKLPQKKINDFCR
SQCPPIKFISADVHGIWSRFLCDFGDEFVLDL
TGEEPKEIFISNITQANPGIVTCLENHPHKLET
GQFLTFREINGMTGLNGSIQQITVISPFSSIGDT
TELEPYLHGGIAVQVKTPTVFFESLERQLKHPK
CLIVDFSNPEAPLEIHTAMLALDQFQEKYSRKP
VGCQQDSEELLKLATSISETLEEKPDVNADIVH
WLSWTAQGFLSPLAAAVGGVASQEVLKAVTG
KFSPLCQWLYLEAADIVESLGKPECEEFPRGDRY
DALRACIGDTLCQKLQNLNIFLVGCGAIGCEM
LKNFALLGVGTSKEKGMITVTDPLIEKSNLN
RQFLFRPHHIQPKSYTAADATLTKINSQIKIDAH
NKVCPTTETIYNDEFYTKQDVIITALDNVEARRY
VD SRCLANLRPLDLSGTMGKGTGHEVIVPHL
TESYN SHRDPEEEIPFCTLKSFPAAIEHTIQ
WARDKFESSF SHKPSLFNKFWQTYSSAEV
LQKIQSGHSLEGCFQ VIKLLSRRPRNWS
QCVELARLKFEKYFNHKALQLL HCFPLD
IRLKDGLFWQSPKRPPSPIKFDLNEPL
HLSFLQNAAKLYATVYCIPEEEDLSADALL
NIL SEVKIQEFKPSNKVVQTDETARKPDH
VPISSD ERNAIFQLEKAILSNEATKSDLQ
MAVLSFEKD DDHNGHIDFITAASNLR
AKMYSIEPADRFKT KRIAGKIIPAIATTT
ATVSGLVALEMIVTGGYP FEAYKNCFL
NLAIPIVVFTEVTRKTKIRNGISFTI
WDRWTVHGKEDFTLLDFINAVKEKYGIE
PTMV VQGVKMLYVPMVPGHAKRLKLT
MHKLVKPTTEK KYVDLTVSFAPDIDG
DEDLPGPPVRYFSDTD

Tag (**bold text**): N-terminal His
Protease cleavage site: TEV (**ENLYFQ**▼**G**)
UBA6 (regular text): Start **bold italics** (amino acid residues 1-1052)
Accession number: NP_060697



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