UBA7 [6His-tagged] E1 Activating Enzyme

Alternate Name: UBE1L

Cat. No.	61-0007-050
Lot. No.	30080

Quantity: 50 µg Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



## **CERTIFICATE OF ANALYSIS Page 1 of 2**

Protein Sequences: Please see page 2

## Background

The enzymes of the ISGylation pathway play a pivotal role in the immnune response. Three classes of enzymes are involved in the process of ISGylation an activating enzyme (E1), conjugating enzymes (E2s) and protein ligases (E3s). UBA7 is a member of the E1 activating enzyme family and cloning of the human gene was first described by Kok et al. (1993). The UBA7 gene has been mapped to chromosome 3p21 by high resolution fluorescence in situ hybridization (Carritt et al. 1992). UBA7 and UBE2L6 have been reported to function as the E1 and E2 enzymes respectively for ISG conjugation forming a thioester intermediate through Cys-131 of UBE2L6 (Takeuchi et al. 2005). UBA7 (Ube1L) knockout (KO) mice are deficient in IS-Gylation and are fertile with no obvious phenotype. (Kim et al. 2006). The expression of UBA7 was found to be reduced in many lung cancer cell lines and although originally thought to be a tumor suppressor gene candidate recent studies have demonstrated that UBA7 does not suppress the development of lung adenoma or thymic lymphoma in a KrasLA2 cancer model (Kok et al. 1993).

### References:

Carritt B, Kok K, van den Berg A, Osinga J, Pilz A, et al. (1992) A gene from human chromosome region 3p21 with reduced expression in small cell lung cancer. *Cancer Res* **52**, 1536-1541.

Kim KI, Yan M, Malakhova O, Luo JK, Shen MF, et al. (2006) Ube1L and protein ISGylation are not essential for alpha/beta interferon signaling. *Mol Cell Biol* **26**, 472-479.

Kok K, Hofstra R, Pilz A, van den Berg A, Terpstra P, et al. (1993) A gene in the chromosomal region 3p21 with greatly reduced expression in lung cancer is similar to the gene for ubiquitinactivating enzyme. *Proc Natl Acad Sci USA* **90**, 6071-6075.

Continued on page 2

# **Physical Characteristics**

Species: human

Source: Insect sf21

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: 116.53 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

# **Quality Assurance**

### Purity:

4-12% gradient SDS-PAGE InstantBlue™ staining Lane 1: MW markers Lane 2: 1 μg His-UBA7

kDa



## Protein Identification:

Confirmed by mass spectrometry.

## E1 Thioester ISG15 Loading Assay:

The activity of His-UBA7 was validated by loading ISG15 onto the active cysteine of His-UBA7. Incubation of the His-UBA7 enzyme in the presence of ISG15 and ATP at  $30^{\circ}$ C was compared at two time points, T<sub>0</sub> and T<sub>10</sub> minutes. Sensitivity of the ISG15/His-UBA7 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

#### Continued from page 1

Takeuchi T, Iwahara S, Saeki Y, Sasajima H, Yokosawa H (2005) Link between the ubiquitin conjugation system and the ISG15 conjugation system: ISG15 conjugation to the UbcH6 ubiquitin E2 enzyme. *J Biochem* **138**, 711-719.

# **Physical Characteristics**

### Continued from page 1

## **Protein Sequence:**

**MSYYHHHHHHDYDIPTT**ENLYFQGAMGS GIORPTSTSSLVAAA**M**DALDASKLL DEELYSROLYVLGSPAMORIOGARVLVS GLQGLGAEVAKNLVLMGVGSLTLHDPHPTCWS DLAAQFLLSEQDLERSRAEASQELLAQLN RAVQVVVHTGDITEDLLLDFQVVVLTAAKLE EQLKVGTLCHKHGVCFLAADTRGLVGQLFCD FGEDFTVQDPTEAEPLTAAIQHISQG SPGILTLRKGANTHYFRDGDLVTFSGIEGM VELNDCDPRSIHVREDGSLEIGDTTTFSRYL RGGAITEVKRPKTVRHKSLDTALLQPHV VAOSSOEVHHAHCLHOAFCALHKFOHLHGR PPQPWDPVDAETVVGLARDLEPLKRTEEE PLEEPLDEALVRTVALSSAGVLSPMVAML GAVAAOEVLKAISRKFMPLDOWLYFDALD CLPEDGELLPSPEDCALRGSRYDGQIAVF GAGFQEKLRRQHYLLVGAGAIGCELLKV FALVGLGAGNSGGLTVVDMDHIERSNLSRQ FLFRSQDVGRPKAEVAAAAARGLNPDLQVI PLTYPLDPTTEHIYGDNFFSRVDGVAAALDS FQARRYVAARCTHYLKPLLEAGTSGTWGSAT VFMPHVTEAYRAPASAAASEDAPYPVCT VRYFPSTAEHTLQWARHEFEELFRLSAET INHHQQAHTSLADMDEPQTLTLLKPVLGVL RVRPONWODCVAWALGHWKLCFHYGIKOLL RHFPPNKVLEDGTPFWSGPKQCPQPLEFDT NQDTHLLYVLAAANLYAQMHGLPGSQD WTALRELLKLLPQPDPQQMAPIFASNLELA SASAEFGPEQQKELNKALEVWSVGPPLKPLM FEKDDDSNFHVDFVVAAASLRCQNYGIP **PVNRAQSKRIVGQIIPAIATTTAAVAGLL** GLELYKVVSGPRPRSAFRHSYLHLAENY LIRYMPFAPAIQTFHHLKWTSWDRLKV PAGQPERTLESLLAHLQEQHGLRVRILLHG SALLYAAGWSPEKOAOHLPLRVTELVOOLT GOAPAPGORVLVLELSCEGDDEDTAFPPLHYEL

Tag (bold text): N-terminal His Protease cleavage site: TEV (<u>ENLYF▼QG</u>) UBA7 (regular text): Start **bold italics** (amino acid residues 1-1012) Accession number: NP\_003326.2



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