ATG7 [6His-tagged]

E1 Activating Enzyme

Alternate Name: APG7, GSA7

Cat. No.	**61-0008-050**	Quantity:	50 µg
Lot. No.	**30081**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

The enzymes of the autophagy pathway play a pivotal role in the degradation of cytoplasmic constituents and organelles. Structures known as autophagosomes sequester portions of the cytoplasm which are degraded by the lysosome and recycled back into the cell (Kuma *et al.* 2004). Three classes of enzymes are involved in autophagy; E1-like activating enzymes, E2-like conjugating enzymes and E3-like ligases. Ubiquitin-like proteins (ubl) Autophagy 12 (ATG12) and ATG8 are activated by ATG7 the E1-like activating enzyme. ATG12 and ATG8 are transferred to two E2-like conjugating enzymes ATG10 and ATG3 respectively. Cloning of the human ATG7 gene was first described by Yuan *et al.* (1999) and ATG7 shares 38% sequence identity to its yeast homologue Apg7. ATG7 can also activate ATG8 mammalian homologues, GABARAP and GATE-16. It forms a homodimer via the C-terminal region that is important for enzyme substrate interaction and E1-E2 complex formation (Komatsu *et al.* 2005). ATG7ΔFAP a mutant form of the ATG7 E1-like activating enzyme which lacks the (Phe-Asp-Pro) FAP motif has been shown to be unable to form an E2 substrate intermediate with ATG3 and the ubl Microtubule-Associated Protein 1, Light Chain 3 (MAPLC3) (Tanida *et al.* 2012). ATG7 has been shown to be involved in a novel pathway in which the inhibition of caspase-8 results in autophagic death induced by receptor-interacting protein (RIP), Jun amino-terminal kinase, and beclin-1 (Yu *et al.* 2004).

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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: 81.34 kDa

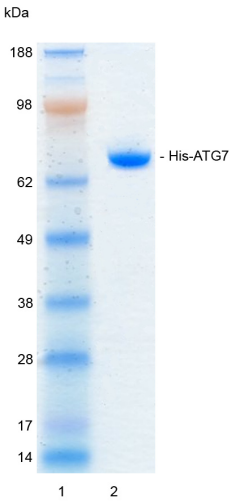
Purity: >95% by InstantBlue™ SDS-PAGE

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

Protein Sequences: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-ATG7



Protein Identification:
Confirmed by mass spectrometry.

E1 Thioester GABARAP Loading Assay:
The activity of His-ATG7 was validated by loading GABARAP onto the active cysteine of His-ATG7. Incubation of the His-ATG7 enzyme in the presence of GABARAP and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the GABARAP/His-ATG7 thioester bond to the reducing agent DTT was confirmed.

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

Lot-specific COA version tracker: v1.0.0

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Background

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References:

Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, *et al.* (2005) Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J Cell Biol* **169**, 425-434.

Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, *et al.* (2004) The role of autophagy during the early neonatal starvation period. *Nature* **432**, 1032-1036.

Tanida I, Yamasaki M, Komatsu M, Ueno T (2012) The FAP motif within human ATG7, an autophagy-related E1-like enzyme, is essential for the E2-substrate reaction of LC3 lipidation. *Autophagy* **8**, 88-97.

Yu L, Alva A, Su H, Dutt P, Freundt E, *et al.* (2004) Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. *Science* **304**, 1500-1502.

Yuan W, Stromhaug PE, Dunn WA, Jr. (1999) Glucose-induced autophagy of peroxisomes in Pichia pastoris requires a unique E1-like protein. *Mol Biol Cell* **10**, 1353-1366.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSYYHHHHHHDYDIPTTENLYFQGA***M***GS
MAAATGDPGLSKLQFAPFSSALDVGFWHEL
TQKKLNEYRLDEAPKDIKGYYYNGDSAGL
PARLTLEFSAFDMSAPTPARCCPAIGTLYNT
NTLESFKTADKKLLLEQAANEIWESIKSG
TALENPVLLNKFLLLTFADLKKYHFYYWF
CYPALCLPESLPLIQGPVGLDQRFSLKQIEA
LECAYDNLCQTEGVTALPYFLIKYDENM
VLVSLLKHYSDFFQGQRTKITIGVYDPCN
LAQYPGWPLRNFLVLAAHRWSSSFQSVEVVC
FRDRTMQGARDVAHSIIFEVKLPEMAFSPDCP
KAVGWEKNQKGGMGPRMVNLSECMDPKRLAES
SVDLNLKLMCWRLVPTLDLDKVVSVKCLLL
GAGTLGCNVARTLMGWGVRHITFVDNAKISYS
NPVRQPLYEFEDCLGGGKPKALAAADRLQKIF
PGVNARGFNMSIPMPGHPVNFSSVTLEQARRD
VEQLEQLIESHDVVFLLMDTRESRWLPAVI
AASKRKLVINAALGFDTFVVMRHGLKKPKQQ
GAGDLCPNHPVASADLLGSSLFANIPGYKLG
CYFCNDVVAPGDSTRDRTLDQQCTVSRPGLA
VIAGALAVELMVSVLQHPEGGYAIASSSDDRM
NEPPTSLGLVPHQIRGFLSRFDNVLPVSLAFD
KCTACSSKVLDQYEREGFNFLAKVFNSSHS
FLEDLTGLTLLHQETQAAEIWDMSDDETI

Tag (**bold text**): N-terminal His
Protease cleavage site: Thrombin (ENLYF▼QG)
ATG7 (regular text): Start ***bold italics*** (amino acid residues 1-706)
Accession number: NP_006386

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