Autophagy Vesicle Elongation (Atg12 Conjugation) Antibody Sampler Kit

HAK21031

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
ATG5 [ET1611-38]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R,Mk	32 kDa
ATG12 [HA721504]	20μl	WB,IF-Cell,FC	H	15 kDa
ATG16L1 [ET7106-65]	20μl	WB,IHC-P	H,M,R	68 kDa
ATG7 [ET1610-53]	20μl	WB,IF-Tissue,IHC-P,IP,FC	H,R	78 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The Autophagy Vesicle Elongation (Atg12 Conjugation) Antibody Sampler Kit provides an economical means of detecting proteins related to autophagy vesicle elongation pathway. The kit contains enough antibody to perform two western blot experiments per primary antibody.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Background

Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of bulk cytoplasmic contents.Autophagy is generally activated by conditions of nutrient deprivation but has also been associated with a number of physiological processes including development, differentiation, neurodegeneration, infection, and cancer.

The molecular machinery of autophagy was largely discovered in yeast and referred to as autophagy-related (Atg) genes.Formation of the autophagosome involves a ubiquitin-like conjugation system in which Atg12 is covalently bound to Atg5 and targeted to autophagosome vesicles.This conjugation reaction is mediated by the ubiquitin E1-like enzyme Atg7 and the E2-like enzyme Atg10.Atg16L1 binds Atg5 of the Atg12-Atg5 conjugate forming an 800 kDa multimeric complex.The Atg12-Atg-5-Atg16L1 complex localizes to pre-autophagosomal membranes where it determines the site of LC3 lipidation and catalyzes the reaction required for the formation of mature autophagosomes.

Database links: UniProt ID: Q9H1Y0, Q99J83, Q3MQ06, O94817, Q676U5, Q8C0J2, O95352, Q641Y5

Images

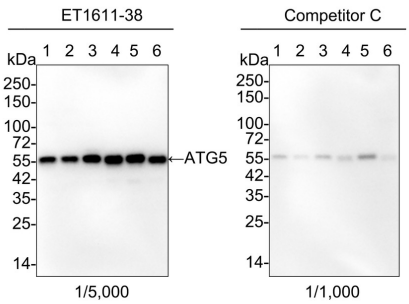

Fig1: Western blot analysis of ATG5 on different lysates with Rabbit anti-ATG5 antibody (ET1611-38) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate (15 µg/Lane)
Lane 2: C2C12 cell lysate (15 µg/Lane)
Lane 3: Neuro-2a cell lysate (15 µg/Lane)
Lane 4: PC-12 cell lysate (15 µg/Lane)
Lane 5: Mouse brain tissue lysate (15 µg/Lane)
Lane 6: Rat brain tissue lysate (15 µg/Lane)

Predicted band size: 32 kDa
Observed band size: 55 kDa

Exposure time: 35 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1611-38) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

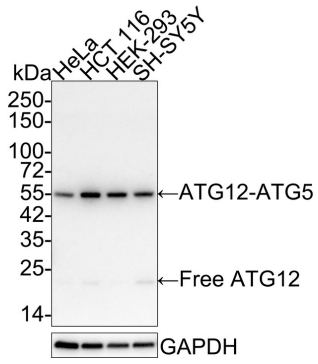

Fig2: Western blot analysis of ATG12 on different lysates with Rabbit anti-ATG12 antibody (HA721504) at 1/1,000 dilution.

Lane 1: HeLa cell lysate, 20 µg/Lane
Lane 2: HCT 116 cell lysate, 20 µg/Lane
Lane 3: HEK-293 cell lysate, 20 µg/Lane
Lane 4: SH-SY5Y cell lysate, 20 µg/Lane

Predicted band size: 15 kDa
Observed band size: 55/20 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721504) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

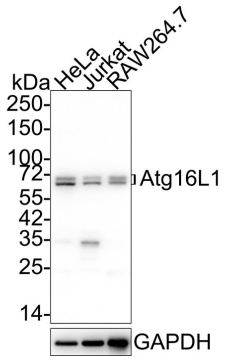

Fig3: Western blot analysis of Atg16L1 on different lysates with Rabbit anti-Atg16L1 antibody (ET7106-65) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: RAW264.7 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 68 kDa
Observed band size: 68/66 kDa

Exposure time: 2 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET7106-65) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Background References

1. Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. Mol Biol Cell. 2008 May;19(5):2092-100.
2. Codogno P, Meijer AJ. Autophagy and signaling: their role in cell survival and cell death. Cell Death Differ. 2005 Nov;12 Suppl 2:1509-18.
3. Levine B, Yuan J. Autophagy in cell death: an innocent convict? J Clin Invest. 2005 Oct;115(10):2679-88.
4. Mizushima N, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Takao T, Natsume T, Ohsumi Y, Yoshimori T. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. J Cell Sci. 2003 May 1;116(Pt 9):1679-88.