Autophagy Vesicle Elongation (LC3 Conjugation) Antibody Sampler Kit

HAK21030

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
MAP1LC3A [ET1609-26]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R	14 kDa
LC3B [ET1701-65]	20μl	WB,IF-Cell,IHC-P,IF-Tissue,IP,mIHC	H,M,R	14/16 kDa
ATG7 [ET1610-53]	20μl	WB,IF-Tissue,IHC-P,IP,FC	H,R	78 kDa
ATG4B [HA721829]	20μl	WB	H,M,R,Mk	44 kDa
ATG4A [HA721010]	20μl	WB,IHC-P,FC,IF-Cell	H,M,R	45 kDa
GABARAP [ET1705-53]	20μl	WB,IHC-P,FC	H,M,R	14 kDa
Apg3 [ET1706-29]	20μl	WB,IHC-P	H,M,R	40 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The Autophagy Vesicle Elongation (LC3 Conjugation) Antibody Sampler Kit provides an economical means of detecting target proteins related to autophagy vesicle elongation pathway. The kit contains enough antibody to perform two western blots per primary.

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 marker Light Chain 3 (LC3) was originally identified as a subunit of microtubule-associated proteins 1A and 1B (termed MAP1LC3).Three human LC3 isoforms (LC3A, LC3B, and LC3C) undergo post-translational modifications during autophagy.Cleavage of LC3 at the carboxy terminus immediately following synthesis yields the cytosolic LC3-I form. During autophagy, LC3-I is converted to LC3-II through lipidation by a ubiquitin-like system involving Atg7 and Atg3 that allows for LC3 to become associated with autophagic vesicles.The presence of LC3 in autophagosomes and the conversion of LC3 to the lower migrating form, LC3-II, have been used as indicators of autophagy.

Numerous mammalian counterparts to yeast Atg proteins have been described, including three Atg8 proteins (GATE-16, GABARAP, and LC3) and four Atg4 homologs (Atg4A/autophagin-2, Atg4B/autophagin-1, Atg4C/autophagin-3, and Atg4D/autophagin-4). The cysteine protease Atg4 is pivotal to autophagosome membrane generation and regulation.GABAA receptor associated protein (GABARAP) is an Atg8 family protein with a key role in autophagy.Processing of GABARAP involves cleavage by Atg4 family members followed by conjugation by the E1 and E2 like enzymes Atg7 and Atg3.

Database links: UniProt ID: Q9H492, Q91VR7, Q6XVN8, Q9GZQ8, Q9CQV6, Q62625, O95352, Q641Y5, Q9Y4P1, Q8BGE6, Q62625, Q8WYN0, Q8C9S8, B1H274, O95166, Q8R3R8, P60517, Q9NT62, Q9CPX6, Q6AZ50

Images

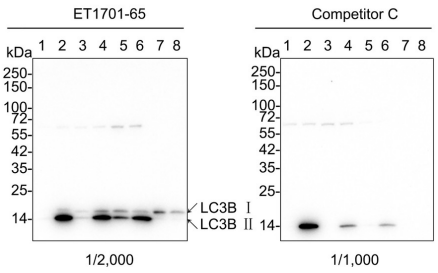

Fig1: Western blot analysis of LC3B on different lysates with Rabbit anti-LC3B antibody (ET1701-65) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 50μM Chloroquine for 18 hours cell lysate
Lane 3: C2C12 cell lysate
Lane 4: C2C12 treated with 50μM Chloroquine for 18 hours cell lysate
Lane 5: C6 cell lysate
Lane 6: C6 treated with 50μM Chloroquine for 18 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 14/16 kDa
Observed band size: 14/16 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 (ET1701-65) at 1/2,000 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.

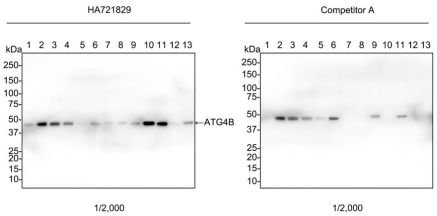

Fig2: Western blot analysis of ATG4B on different lysates with Rabbit anti-ATG4B antibody (HA721829) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: 293T cell lysate
Lane 4: MCF7 cell lysate
Lane 5: K-562 cell lysate
Lane 6: COS-1 cell lysate
Lane 7: NIH/3T3 cell lysate
Lane 8: C2C12 cell lysate
Lane 9: PC-12 cell lysate
Lane 10: Mouse brain tissue lysate
Lane 11: Rat brain tissue lysate
Lane 12: Mouse kidney tissue lysate
Lane 13: Mouse colon tissue lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 44 kDa
Observed band size: 47 kDa

Exposure time: 42 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 (HA721829) at 1/2,000 dilution and competitor's antibody at 1/2,000 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.

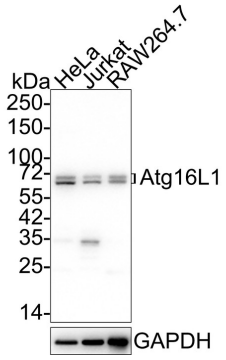

Fig3: Western blot analysis of Apg7 on different lysates with Rabbit anti-Apg7 antibody (ET1610-53) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: THP-1 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 78 kDa
Observed band size: 70 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 (ET1610-53) at 1/2,000 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.

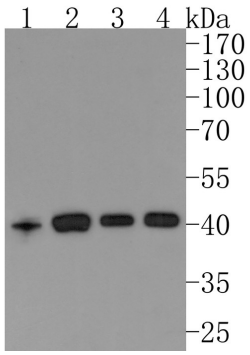

Fig4: Western blot analysis of ATG4A on different lysates with Rabbit anti-ATG4A antibody (HA721010) at 1/500 dilution.

Lane 1: K562 cell lysate, 10 µg/Lane
Lane 2: Jurkat cell lysate, 10 µg/Lane
Lane 3: Mouse kidney tissue lysate, 20 µg/Lane
Lane 4: Rat kidney tissue lysate, 20 µg/Lane

Predicted band size: 45 kDa
Observed band size: 41 kDa

Exposure time: 2 minutes;

10% 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 (HA721010) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Background References

1. Sou YS, Waguri S, Iwata J, Ueno T, Fujimura T, Hara T, Sawada N, Yamada A, Mizushima N, Uchiyama Y, Kominami E, Tanaka K, Komatsu M. The Atg8 conjugation system is indispensable for proper development of autophagic isolation membranes in mice. Mol Biol Cell. 2008 Nov;19(11):4762-75.
2. Levine B, Yuan J. Autophagy in cell death: an innocent convict? J Clin Invest. 2005 Oct;115(10):2679-88.
3. 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.
4. Wu J, Dang Y, Su W, Liu C, Ma H, Shan Y, Pei Y, Wan B, Guo J, Yu L. Molecular cloning and characterization of rat LC3A and LC3B--two novel markers of autophagosome. Biochem Biophys Res Commun. 2006 Jan 6;339(1):437-42.