Autophagosome Marker Antibody Sampler Kit

HAK21022

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
ATG12 [HA721504]	20μl	WB,IF-Cell,FC	H	15 kDa
MAP1LC3A [ET1611-37]	20μl	WB,IF-Cell,IHC-P,IP	H,M,R	14 kDa
LC3B [ET1701-65]	20μl	WB,IF-Cell,IHC-P,IF-Tissue,IP,mIHC	H,M,R	14/16 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The Autophagosome Marker Sample Antibody Sampler Kit provides an economical means to investigate the accumulation of autophagosomes within the cell. The kit contains enough primary and secondary antibodies to perform two western blots 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 .

Autophagy marker Light Chain 3 (LC3) was originally identified as a subunit of microtubule-associated proteins 1A and 1B (termed MAP1LC3) and subsequently found to contain similarity to the yeast protein Apg8/Aut7/Cvt5 that is critical for autophagy. 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 .

Database links: UniProt ID: O94817, Q9H492, Q91VR7, Q6XVN8, Q9GZQ8, Q9CQV6, Q62625

Images

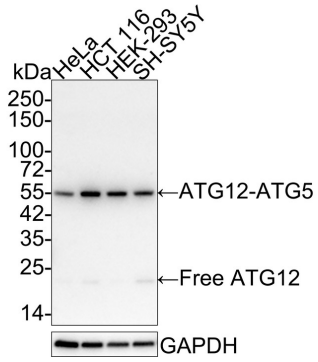

Fig1: Western blot analysis of ATG12 on different lysates with Rabbit anti-ATG12 antibody (HA721504) at 1/1,000 dilution.

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.

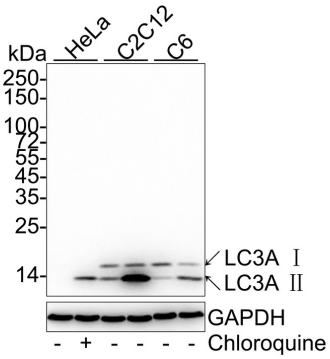

Fig2: Western blot analysis of MAP1LC3A on different lysates with Rabbit anti-MAP1LC3A antibody (ET1611-37) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HeLa treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 3: C2C12 cell lysate (20 µg/Lane)
Lane 4: C2C12 treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 6: C6 treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)

Predicted band size: 14 kDa
Observed band size: 14/16 kDa

Exposure time: 24 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 (ET1611-37) 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.

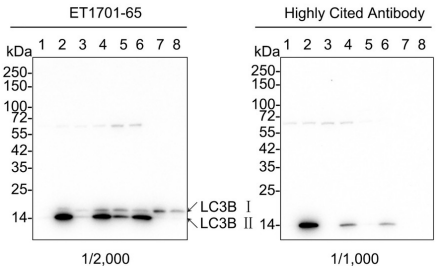

Fig3: Western blot analysis of LC3B on different lysates with Rabbit anti-LC3B antibody (ET1701-65) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HeLa treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 3: C2C12 cell lysate (20 µg/Lane)
Lane 4: C2C12 treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 6: C6 treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 7: mouse brain tissue lysate (20 µg/Lane)
Lane 8: rat brain tissue lysate (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.

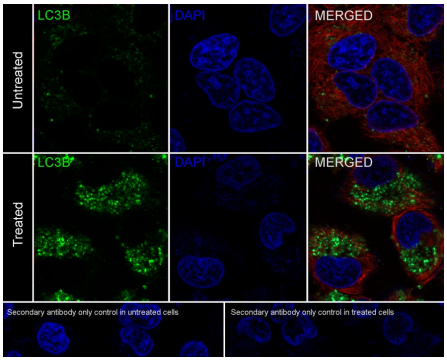

Fig4: Immunocytochemistry analysis of Hela cells treated with or without 50µM Chloroquine for 24 hours labeling LC3B with Rabbit anti-LC3B antibody (ET1701-65) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-LC3B antibody (ET1701-65) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

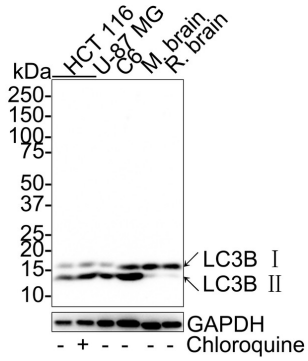

Fig5: Western blot analysis of LC3B on different lysates with Rabbit anti-LC3B antibody (ET1701-65) at 1/1,000 dilution.

Lane 1: HCT 116 cell lysate (20 µg/Lane)
Lane 2: HCT 116 treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 3: U-87 MG cell lysate (20 µg/Lane)
Lane 4: C6 cell lysate (20 µg/Lane)
Lane 5: Mouse brain tissue lysate (20 µg/Lane)
Lane 6: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 14/16 kDa
Observed band size: 14/16 kDa

Exposure time: 26 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 (ET1701-65) 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.

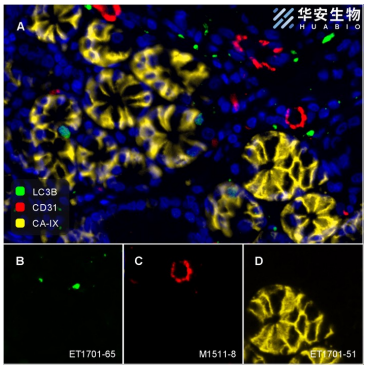

Fig6: Fluorescence multiplex immunohistochemical analysis of human gastric cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-LC3B (ET1701-65, Green), anti-CD31 (M1511-8, Red) and anti-CA-IX (ET1701-51, Yellow) on human gastric cancer. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1701-65 (1/100 dilution), M1511-8 (1/2,000 dilution) and ET1701-51 (1/100 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Background References

1. Reggiori, F. and Klionsky, D.J. (2002) Eukaryot Cell 1, 11-21.
2. Codogno, P. and Meijer, A.J. (2005) Cell Death Differ 12 Suppl 2, 1509-18.
3. Levine, B. and Yuan, J. (2005) J Clin Invest 115, 2679-88.
4. Mizushima, N. et al. (1998) J Biol Chem 273, 33889-92.
5. Mizushima, N. et al. (1998) Nature 395, 395-8.
6. Suzuki, K. et al. (2001) EMBO J 20, 5971-81.
7. Tanida, I. et al. (1999) Mol Biol Cell 10, 1367-79.
8. Shintani, T. et al. (1999) EMBO J 18, 5234-41.
9. Mann, S.S. and Hammarback, J.A. (1994) J Biol Chem 269, 11492-7.
10. Lang, T. et al. (1998) EMBO J 17, 3597-607.
11. Kabeya, Y. et al. (2000) EMBO J 19, 5720-8.
12. Wu, J. et al. (2006) Biochem Biophys Res Commun 339, 437-42.