Autophagy Essentials Antibody Sampler Kit

HAK21114

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Beclin 1 [HA721216]	20μl	WB,IHC-P,IF-Cell	H,M,R	52 kDa
LC3B [ET1701-65]	20μl	WB,IF-Cell,IHC-P,IF-Tissue,IP,mIHC	H,M,R	14/16 kDa
SQSTM1 / p62 [HA721171]	20μl	WB,IHC-P,IF-Cell,FC,IF-Tissue,IHC-Fr	H,M,R	48 kDa
ATG5 [ET1611-38]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R,Mk	32 kDa
LAMP1 [HA722827]	20μl	WB,IHC-P,IF-Tissue,IHC-Fr	H,M,R	44 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The Autophagy Essentials Antibody Sampler Kit includes enough antibodies to perform two western blot experiments with each 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 highly dynamic process consisting of the following three steps: (1) autophagosome formation, (2) autophagosome-lysosome fusion, and (3) degradation. It can be induced by multiple signaling pathways related to various triggers including nutrient deprivation, growth factor signaling, and cellular stress. The process of autophagosome formation proceeds through the steps of initiation, nucleation, elongation, closure, and ultimately fusion, each of which is regulated by various ATG proteins.

The ideal approach for measuring autophagy is to assess autophagic flux, which represents the rate of degradation of the autophagic pathway. The most widely used method for measuring autophagic flux is to detect the processing of the autophagosomal membrane protein, LC3. Analyzing autophagy substrates such as p62/SQSTM1 is often recommended in addition to measuring LC3-II turnover for accurate assessment of autophagic flux. The fusion of autophagosomes with lysosomes can be monitored by analyzing the autophagosomal marker LC3 and the lysosomal marker, LAMP simultaneously.

Database links: UniProt ID: Q14457, O88597, Q91XJ1, Q9GZQ8, Q9CQV6, Q62625, Q13501, Q64337, O08623, Q9H1Y0, Q99J83, Q3MQ06, P11279, P11438, P14562

Images

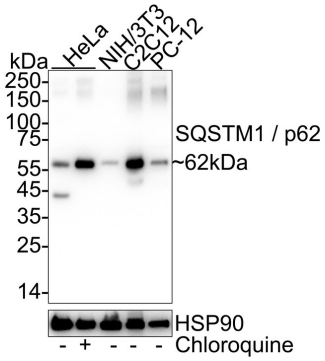

Fig1: Western blot analysis of SQSTM1 / p62 on different lysates with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/10,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 50μM Chloroquine for 18 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: C2C12 cell lysate
Lane 5: PC-12 cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 48 kDa
Observed band size: 62 kDa

Exposure time: 2 seconds; ECL: K1801;
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 (HA721171) at 1/10,000 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.

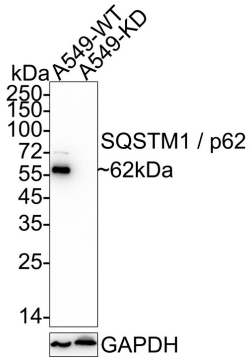

Fig2: Western blot analysis of SQSTM1 / p62 on different lysates with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/2,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si SQSTM1 / p62 cell lysate

Lysates/proteins at 10 μg/Lane.

Predicted band size: 48 kDa
Observed band size: 62 kDa

Exposure time: 17 seconds; ECL: K1801;

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 (HA721171) 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.

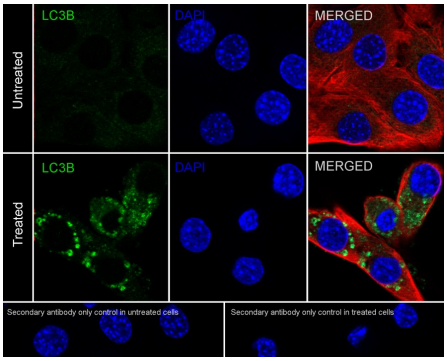

Fig3: Immunocytochemistry analysis of C2C12 cells treated with or without 50μM Chloroquine for 24 hours labeling LC3B with Rabbit anti-LC3B antibody (ET1701-65) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-LC3B antibody (ET1701-65) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. 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.

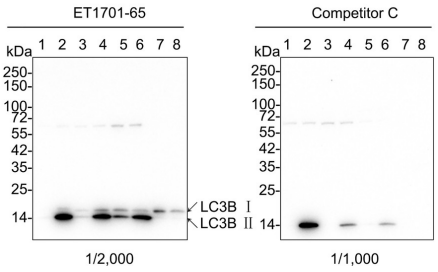

Fig4: Western blot analysis of LC3B on different lysates with Rabbit anti-LC3B antibody (ET1701-65) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

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
Lane 7: mouse brain tissue lysate
Lane 8: rat brain tissue 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.

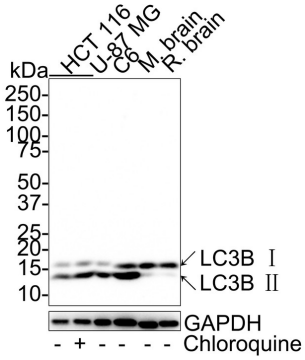

Fig5: Western blot analysis of LC3B on different lysates with Rabbit anti-LC3B antibody (ET1701-65) at 1/1,000 dilution.

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

Lysates/proteins at 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.

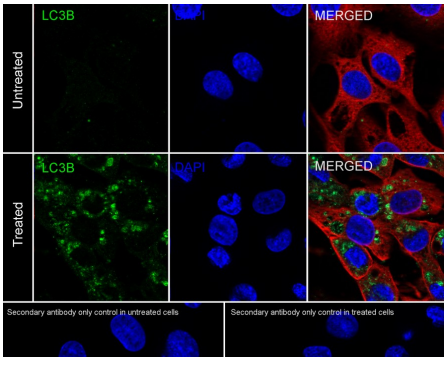

Fig6: Immunocytochemistry analysis of C6 cells treated with or without 50μM Chloroquine for 24 hours labeling LC3B with Rabbit anti-LC3B antibody (ET1701-65) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-LC3B antibody (ET1701-65) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. 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.

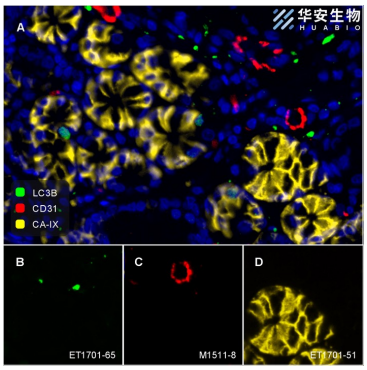

Fig7: Fluorescence multiplex immunohistochemical analysis of human gastric cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-LC3B (ET1701-65, Green), anti-CD31 (M1511-8, Red) and anti-CA-IX (ET1701-51, Yellow) on human gastric cancer. HRP Conjugated UltraPolymer 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.

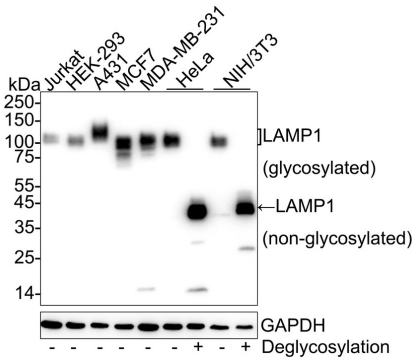

Fig8: Western blot analysis of LAMP1 on different lysates with Rabbit anti-LAMP1 antibody (HA722827) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: A431 cell lysate
Lane 4: MCF7 cell lysate
Lane 5: MDA-MB-231 cell lysate
Lane 6: HeLa cell lysate
Lane 7: HeLa cell lysate treated with deglycosylation
Lane 8: NIH/3T3 cell lysate
Lane 9: NIH/3T3 cell lysate treated with deglycosylation

Lysates/proteins at 20 µg/Lane.

Predicted band size: 44 kDa
Observed band size: 120/44 kDa

Exposure time: 21 seconds; ECL: K1801;

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 (HA722827) 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.

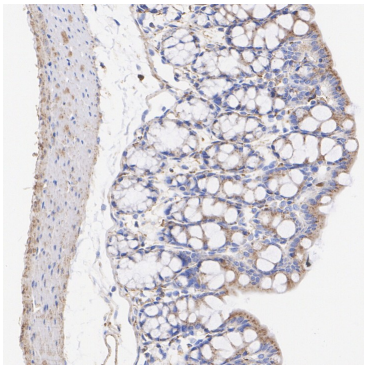

Fig9: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-LAMP1 antibody (HA722827) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722827) at 1/10,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

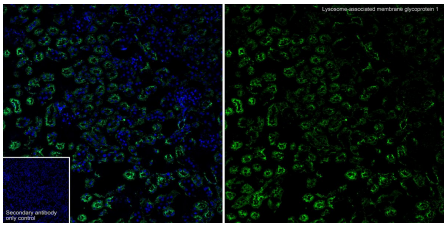

Fig10: Immunofluorescence analysis of frozen mouse kidney tissue with Rabbit anti-LAMP1 antibody (HA722827) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722827, green) at 1/1,000 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Liu S, Yao S, Yang H, Liu S, Wang Y. Autophagy: Regulator of cell death. Cell Death Dis. 2023 Oct 4;14(10):648.
2. Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal. 2014 Jan 20;20(3):460-73.
3. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. J Pathol. 2010 May;221(1):3-12.