ER Stress-induced Autophagy Antibody Sampler Kit

HAK21029

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
GRP78 / BIP [HA601076]	20μl	WB,IHC-P,IF-Cell	H,M,R	72 kDa
EIF2S1 [HA722112]	20μl	WB,IF-Cell,IHC-P,FC	H,M,R,Mk	36 kDa
Phospho-EIF2S1 (S51) [ET1603-14]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R	36 kDa
ATG12 [HA721504]	20μl	WB,IF-Cell,FC	H	15 kDa
Beclin 1 [HA721216]	20μl	WB,IHC-P,IF-Cell	H,M,R	52 kDa
JNK1+JNK2+JNK3 [ET1601-28]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP	H,M,R	48/53 kDa
Phospho-JNK1/2/3 (T183 + T183 + T221) [ET1609-42]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC,IHC-Fr	H,M,R	48/53 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	
HRP-Goat Anti-Mouse IgG (H+L) [HA1006]	100μl	WB,ELISA,IHC-P	M	

Description: The ER Stress-induced Antibody Sampler Kit contains reagents to investigate ER stress-induced signaling within the cell. The kit contains enough primary antibodies to perform four 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

The endoplasmic reticulum (ER) is an organelle with essential biosynthetic and signaling functions in eukaryotic cells.ER stress activates an intracellular signaling transduction pathway called unfolded protein response (UPR) and autophagy to avoid cell death .

One of the chaperones aiding in proper protein folding is Binding immunoglobulin Protein (BiP). BiP works by binding to misfolded proteins to prevent them from forming aggregates and assists in proper refolding.-Formation of the autophagosome involves a ubiquitin-like conjugation system in which Atg12 is covalently bound to Atg5 and targeted to autophagosome vesicles. One of the proteins critical to autophagy process is Beclin-1, the mammalian orthologue of the yeast autophagy protein Apg6/Vps30. Phosphorylation of the eukaryotic initiation factor 2 (eIF2) α subunit is a well-documented mechanism to downregulate protein synthesis under a variety of stress conditions. eIF2 binds GTP and Met-tRNAi and transfers Met-tRNA to the 40S subunit to form the 43S preinitiation complex.The IRE1, a transmembrane serine/threonine kinase,through its kinase activity activates SAPK/JNK in the early stage of ER stress in order to induce autophagosome formation .

Database links: UniProt ID: P11021, P20029, P06761, P05198, Q6ZWX6, P68101, P05198, Q6ZWX6, P68101, O94817, Q14457, O88597, Q91XJ1, P45983, P45984, P53779, Q61831, Q91Y86, Q9WTU6, P49185, P49186, P49187, P45983, P45984, P53779, Q61831, Q91Y86, Q9WTU6, P49185, P49186, P49187

Images

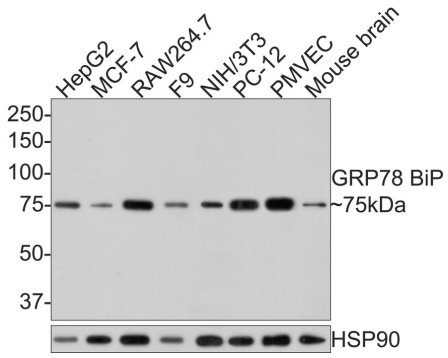

Fig1: Western blot analysis of GRP78 BiP on different lysates with Mouse anti-GRP78 BiP antibody (HA601076) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate (10 µg/Lane)
Lane 2: MCF-7 cell lysate (10 µg/Lane)
Lane 3: RAW264.7 cell lysate (10 µg/Lane)
Lane 4: F9 cell lysate (10 µg/Lane)
Lane 5: NIH/3T3 cell lysate (10 µg/Lane)
Lane 6: PC-12 cell lysate (10 µg/Lane)
Lane 7: PMVEC cell lysate (10 µg/Lane)
Lane 8: Mouse brain tissue lysate (20 µg/Lane)

Predicted band size: 72 kDa
Observed band size: 75 kDa

Exposure time: 1 minute;

8% 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 (HA601076) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

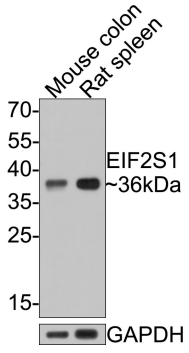

Fig2: Western blot analysis of EIF2S1 on different lysates with Rabbit anti-EIF2S1 antibody (HA500385) at 1/500 dilution.

Lane 1: Mouse colon tissue lysate, 20 µg/Lane
Lane 2: Rat spleen tissue lysate, 20 µg/Lane

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 30 seconds;

12% 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 (HA500385) 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 1:300,000 dilution was used for 1 hour at room temperature.

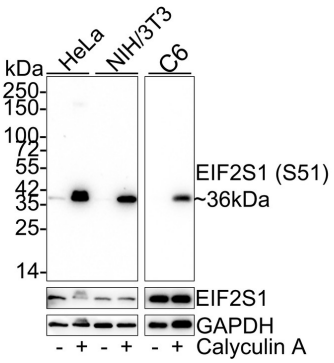

Fig3: Western blot analysis of Phospho-EIF2S1 (S51) on different lysates with Rabbit anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/2,000 dilution.

Lane 1: HeLa whole cell lysate (15 µg/Lane)
Lane 2: HeLa treated with 50nM Calyculin A for 3 hours whole cell lysate (15 µg/Lane)
Lane 3: NIH/3T3 whole cell lysate (15 µg/Lane)
Lane 4: NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate (15 µg/Lane)
Lane 5: C6 whole cell lysate (20 µg/Lane)
Lane 6: C6 treated with 100nM Calyculin A for 30 minutes whole cell lysate (20 µg/Lane)

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: Lane 1-4: 2 minutes; Lane 5-6: 23 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 (ET1603-14) 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.

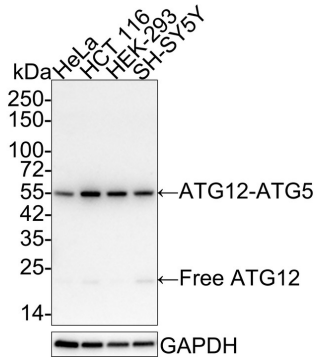

Fig4: Western blot analysis of ATG12 on different lysates with Rabbit anti-ATG12 antibody (HA721504) at 1/1,000 dilution.

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.

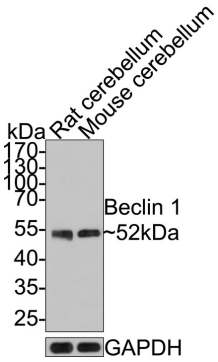

Fig5: Western blot analysis of Beclin 1 on different lysates with Rabbit anti-Beclin 1 antibody (HA721216) at 1/500 dilution.

Lane 1: Rat cerebellum tissue lysate, 20 µg/Lane
Lane 2: Mouse cerebellum tissue lysate, 20 µg/Lane

Predicted band size: 52 kDa
Observed band size: 52 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 (HA721216) 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 1:300,000 dilution was used for 1 hour at room temperature.

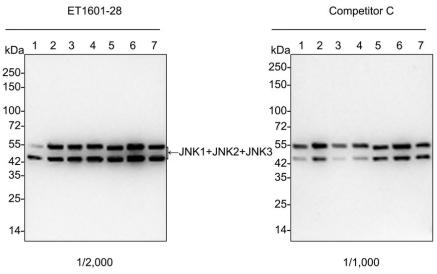

Fig6: Western blot analysis of JNK1+JNK2+JNK3 on different lysates with Rabbit anti-JNK1+JNK2+JNK3 antibody (ET1601-28) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate, 15 µg/Lane
Lane 2: HEK-293 cell lysate, 15 µg/Lane
Lane 3: Jurkat cell lysate, 15 µg/Lane
Lane 4: MCF7 cell lysate, 15 µg/Lane
Lane 5: NIH/3T3 cell lysate, 15 µg/Lane
Lane 6: PC-12 cell lysate, 15 µg/Lane
Lane 7: C6 cell lysate, 15 µg/Lane

Predicted band size: 48/53 kDa
Observed band size: 48/53 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 (ET1601-28) 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.

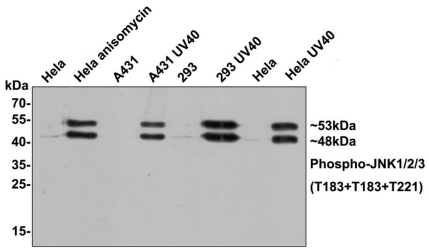

Fig7: Western blot analysis of Phospho-JNK1/2/3(T183+T183+T221) on different lysates with Rabbit anti-Phospho-JNK1/2/3(T183+T183+T221) antibody (ET1609-42) at 1/1,000 dilution.

Lane 1: Hela cell lysate, untreated, 10 µg/Lane
Lane 2: Hela cell lysate, treated with Anisomycin, 10 µg/Lane
Lane 3: A431 cell lysate, untreated, 10 µg/Lane
Lane 4: A431 cell lysate, treated with UV40, 10 µg/Lane
Lane 5: 293 cell lysate, untreated, 10 µg/Lane
Lane 6: 293 cell lysate, treated with UV40, 10 µg/Lane
Lane 7: Hela cell lysate, untreated, 10 µg/Lane
Lane 8: Hela cell lysate, treated with UV40, 10 µg/Lane

Predicted band size: 48/53 kDa
Observed band size: 48/53 kDa

Exposure time: 2 minutes;

12% 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 (ET1609-42) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

Background References

1. Verfaillie T, Salazar M, Velasco G, Agostinis P. Linking ER Stress to Autophagy: Potential Implications for Cancer Therapy. Int J Cell Biol. 2010;2010:930509.
2. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, Murakami T, Taniguchi M, Tanii I, Yoshinaga K, Shiosaka S, Hammarback JA, Urano F, Imaizumi K. Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol. 2006 Dec;26(24):9220-31.
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.
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