α-Synuclein ER Stress Antibody Sampler Kit

HAK21120

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Alpha-Synuclein [HA723035]	20μl	WB,IHC-P,IHC-Fr,IF-Tissue	H,M,R	14 kDa
GBA [ET1703-32]	20μl	WB,IHC-P	H,M,R	60 kDa
Calnexin [ET1611-86]	20μl	WB,IP,IHC-P	H,M,R	68 kDa
GRP78 / BIP [HA722202]	20μl	WB,IHC-P,IF-Tissue	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
DDIT3 [HA722854]	20μl	WB,IF-Cell,FC	H,M,R	19 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The α-Synuclein ER Stress Antibody Sampler Kit provides an economical means to detect and evaluate α-Synuclein induced ER stress within the cell. The kit contains enough antibodies to perform two western blot experiments with each primary antibody.

Storage Buffer: PBS (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

Secretory and transmembrane proteins are synthesized on polysomes and translocate into the endoplasmic reticulum (ER) where they are often modified by the formation of disulfide bonds, amino-linked glycosylation and folding. ER homeostasis disruptions lead to the accumulation of unfolded proteins. The ER has developed an adaptive mechanism called the unfolded protein response (UPR) to counteract compromised protein folding.

The protein kinase-like endoplasmic reticulum kinase (PERK) eIF2α kinase is an ER resident transmembrane protein that couples ER stress signals to translation inhibition. ER stress increases PERK activity, which phosphorylates eIF2α to reduce protein translation. During ER stress, the level of CHOP expression is also elevated and CHOP functions to mediate programmed cell death. α-Synuclein, a 140 amino acid protein expressed abundantly in the brain, is a major component of aggregates found in Lewy bodies.Recent evidence suggests that aggregation of α-Synuclein induces ER stress while also reducing the ability of neurons to respond to protein misfolding through activation of the UPR. Formation of the autophagosome involves a ubiquitin-like conjugation system in which Atg12 is covalently bound to Atg5 and targeted to autophagosome vesicles.

Database links: UniProt ID: P37840, O55042, P37377, P04062, P17439, 684536, P27824, P35564, P35565, P11021, P20029, P06761, P05198, Q6ZWX6, P68101, P05198, Q6ZWX6, P68101, O94817, P35638, P35639, Q62857

Images

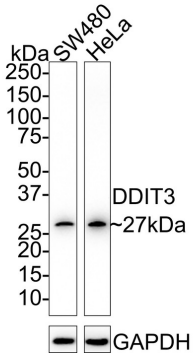

Fig1: Western blot analysis of DDIT3 on different lysates with Rabbit anti-DDIT3 antibody (ET1703-05) at 1/5,000 dilution.

Lane 1: SW480 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)

Predicted band size: 19 kDa
Observed band size: 27 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 (ET1703-05) at 1/5,000 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.

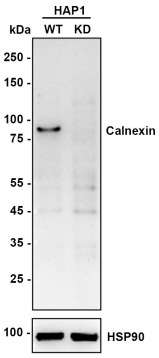

Fig2: Western blot analysis of Calnexin on different lysates with Rabbit anti-Calnexin antibody (ET1611-86) at 1/1,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Calnexin KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 68 kDa
Observed band size: 90 kDa

Exposure time: 180 seconds; ECL: K1802;

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 (ET1611-86) 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.

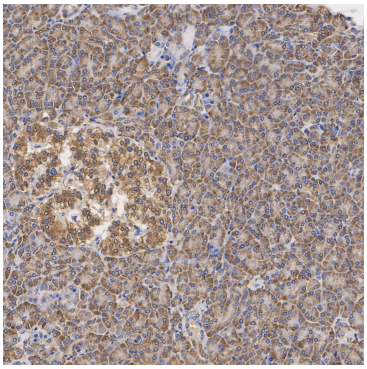

Fig3: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-Calnexin antibody (ET1611-86) at 1/2,000 dilution.

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 (ET1611-86) at 1/2,000 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.

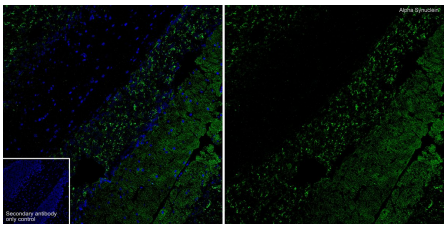

Fig4: Immunofluorescence analysis of frozen mouse cerebellum tissue with Rabbit anti-Alpha-Synuclein antibody (HA723035) at 1/500 dilution.

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 (HA723035, green) at 1/500 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).

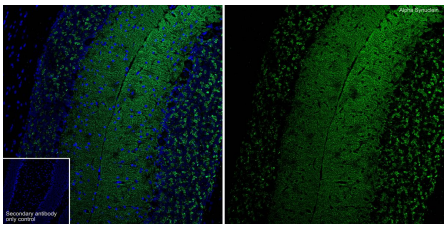

Fig5: Immunofluorescence analysis of frozen rat cerebellum tissue with Rabbit anti-Alpha-Synuclein antibody (HA723035) at 1/500 dilution.

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 (HA723035, green) at 1/500 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).

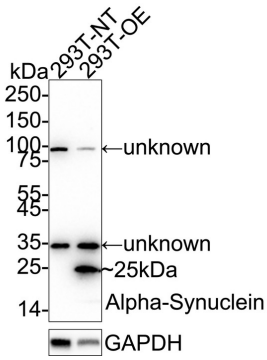

Fig6: Western blot analysis of Alpha-Synuclein on different lysates with Rabbit anti-Alpha-Synuclein antibody (HA723035) at 1/2,000 dilution.

Lane 1: 293T transfected with empty control cell lysate
Lane 2: 293T transfected with Alpha-Synuclein cell lysate

Lysates/proteins at 15 µg/Lane.
Predicted band size: 14 kDa
Observed band size: 25 kDa
Exposure time: 3 minutes; 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 (HA723035) 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.

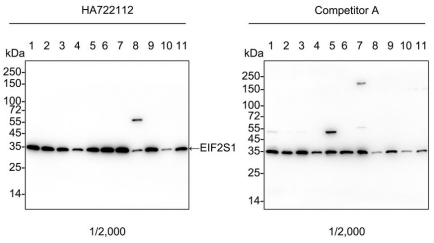

Fig7: Western blot analysis of EIF2S1 on different lysates with Rabbit anti-EIF2S1 antibody (HA722112) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: HepG2 cell lysate
Lane 3: HeLa cell lysate
Lane 4: COS-1 cell lysate
Lane 5: A549 cell lysate
Lane 6: RAW264.7 cell lysate
Lane 7: C6 cell lysate
Lane 8: Mouse kidney tissue lysate
Lane 9: Mouse spleen tissue lysate
Lane 10: Rat kidney tissue lysate
Lane 11: Rat spleen tissue lysate

Lysates/proteins at 10 µg/Lane.
Predicted band size: 36 kDa
Observed band size: 36 kDa
Exposure time: 59 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 (HA722112) 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.

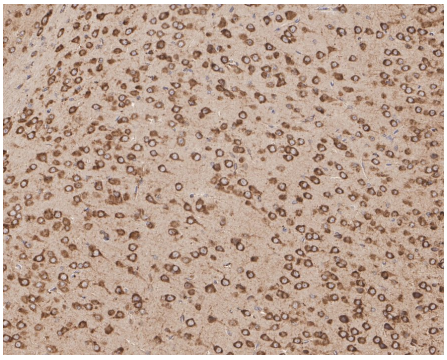

Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-GRP78 / BIP antibody (HA722202) at 1/1,000 dilution.

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 (HA722202) at 1/1,000 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.

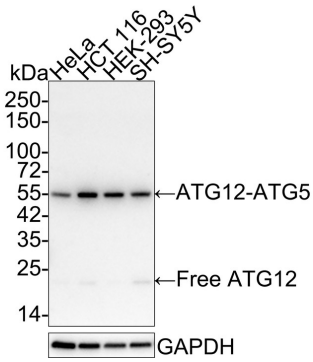

Fig9: Western blot analysis of ATG12 on different lysates with Rabbit anti-ATG12 antibody (HA721504) at 1/1,000 dilution.

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

Lysates/proteins at 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.

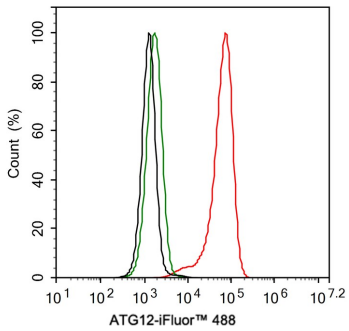

Fig10: Flow cytometric analysis of HCT 116 cells labeling ATG12.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721504, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

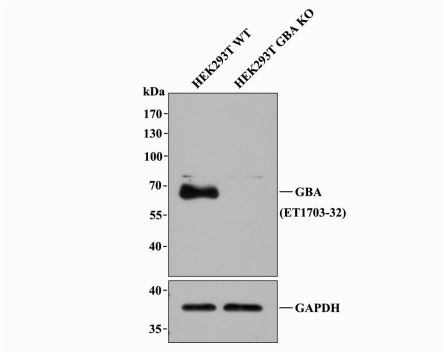

Fig11: All lanes: Western blot analysis of GBA with anti-GBA antibody [JM10-76] (ET1703-32) at 1:1,000 dilution.
Lane 1: Wild-type HEK293T whole cell lysate (20 µg).
Lane 2: GBA knockout HEK293T whole cell lysate (20 µg).

ET1703-32 was shown to specifically react with GBA in wild-type HEK293T cells. No band was observed when GBA knockout sample was tested. Wild-type and GBA knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1703-32, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH , ET1601-4, 1/10,000) was used in 5% BSA 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.

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

Background References

1. Kuo SH, Tasset I, Cheng MM, Diaz A, Pan MK, Lieberman OJ, Hutten SJ, Alcalay RN, Kim S, Ximénez-Embún P, Fan L, Kim D, Ko HS, Yacoubian T, Kanter E, Liu L, Tang G, Muñoz J, Sardi SP, Li A, Gan L, Cuervo AM, Sulzer D. Mutant glucocerebrosidase impairs α-synuclein degradation by blockade of chaperone-mediated autophagy. Sci Adv. 2022 Feb 11;8(6):eabm6393.

2. Hou X, Watzlawik JO, Fiesel FC, Springer W. Autophagy in Parkinson's Disease. J Mol Biol. 2020 Apr 3;432(8):2651-2672.