ER Homeostasis Antibody Sampler Kit

HAK21038

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
FAM134B [HA721752]	20μl	WB,IF-Cell	H,M,R	55 kDa
CCPG1 [HA500520]	20μl	WB,FC	H,M	87 kDa
XBP1 [ET1703-23]	20μl	WB,IHC-P,IF-Cell,IF-Tissue,FC	H,M	29 kDa
ATF6 [HA722722]	20μl	WB,IHC-P,ChIP	H,M,R	75 kDa
Phospho-EIF2S1 (S51) [ET1603-14]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R	36 kDa
PERK [HA721510]	20μl	WB	H	125 kDa
ATF4 [ET1612-37]	20μl	WB,IF-Cell,IF-Tissue,IHC-P	H,M,R	39 kDa
IRE1 [HA723225]	20μl	WB	H,M,R,Mk	110 kDa
GRP78 / BIP [HA601076]	20μl	WB,IHC-P,IF-Cell	H,M,R	72 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 Homeostasis Antibody Sampler Kit provides an economical means of detecting proteins involved in ER homeostasis by regulating ER stress and ER-phagy. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

Storage Buffer: 1*TBS (pH7.4), 0.1% 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 a large organelle extending from the nuclear envelope to the plasma membrane with diversity in structure and function. When demands on protein processing exceeds capabilities, cells trigger an adaptive mechanism called the unfolded protein response (UPR) which is largely controlled by the activities of three pathways: PERK, IRE1α, and ATF-6. The ER chaperone protein BiP is recruited to unfolded proteins in the ER lumen and its dissociation from PERK, IRE1α, and ATF-6 leads to their activation.

FAM134B was the first ER-phagy receptor discovered. Loss of FAM134B can sensitize cells to apoptosis when challenged by nutrient deprivation or ER stress stimuli. CCPG1 is another ER-phagy cargo receptor that associates with FIP200, a component of the ULK1 complex facilitating ER trafficking to autophagosomes.Taken together, signaling from the UPR and ER-phagy help regulate ER homeostasis

Database links: UniProt ID: Q9H6L5, Q8VE91, Q5FVM3, Q9ULG6, Q640L3, P17861, O35426, P18850, 226641, 304962, P05198, Q6ZWX6, P68101, Q9NZJ5, P18848, Q06507, Q9ES19, O75460, Q9EQY0, 498013, P11021, P20029, P06761

Images

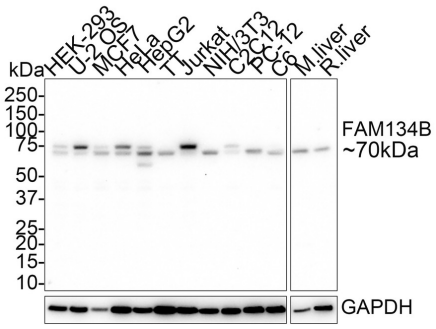

Fig1: Western blot analysis of FAM134B on different lysates with Rabbit anti-FAM134B antibody (HA721752) at 1/1,000 dilution.

Lane 1: HEK-293 cell lysate (30 µg/Lane)
Lane 2: U-2 OS cell lysate (30 µg/Lane)
Lane 3: MCF7 cell lysate (30 µg/Lane)
Lane 4: HeLa cell lysate (30 µg/Lane)
Lane 5: HepG2 cell lysate (30 µg/Lane)
Lane 6: TT cell lysate (30 µg/Lane)
Lane 7: Jurkat cell lysate (30 µg/Lane)
Lane 8: NIH/3T3 cell lysate (30 µg/Lane)
Lane 9: C2C12 cell lysate (30 µg/Lane)
Lane 10: PC-12 cell lysate (30 µg/Lane)
Lane 11: C6 cell lysate (30 µg/Lane)
Lane 12: Mouse liver tissue lysate (30 µg/Lane)
Lane 13: Rat liver tissue lysate (30 µg/Lane)

Predicted band size: 55 kDa
Observed band size: 70 kDa

Exposure time: 6 minutes 20 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 (HA721752) 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.

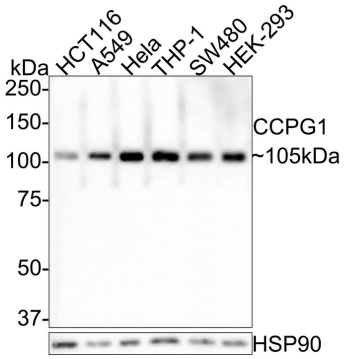

Fig2: Western blot analysis of CCPG1 on different lysates with Rabbit anti-CCPG1 antibody (HA500520) at 1/1,000 dilution.

Lane 1: HCT116 cell lysate
Lane 2: A549 cell lysate
Lane 3: Hela cell lysate
Lane 4: THP-1 cell lysate
Lane 5: SW480 cell lysate
Lane 6: HEK-293 cell lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 87 kDa
Observed band size: 105 kDa

Exposure time: 2 minutes;

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 (HA500520) 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.

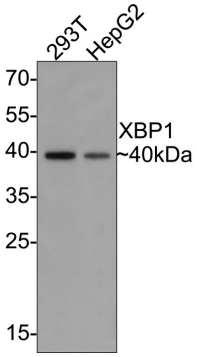

Fig3: Western blot analysis of XBP1 on different lysates with Rabbit anti-XBP1 antibody (ET1703-23) at 1/500 dilution.

Lane 1: 293T cell lysate
Lane 2: HepG2 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 29 kDa
Observed band size: 40 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 (ET1703-23) 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.

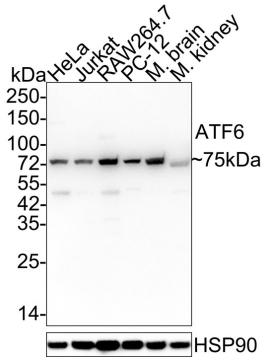

Fig4: Western blot analysis of ATF6 on different lysates with Mouse anti-ATF6 antibody (HA601321) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: RAW264.7 cell lysate (20 µg/Lane)
Lane 4: PC-12 cell lysate (20 µg/Lane)
Lane 5: Mouse brain tissue lysate (40 µg/Lane)
Lane 6: Mouse kidney tissue lysate (40 µg/Lane)

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

Exposure time: 1 minute 21 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 (HA601321) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

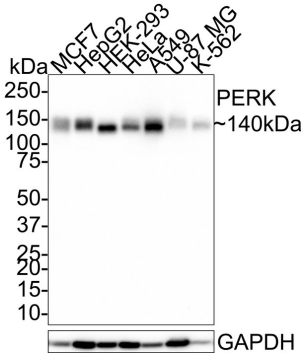

Fig5: Western blot analysis of PERK on different lysates with Rabbit anti-PERK antibody (HA721510) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate (20 µg/Lane)
Lane 2: HepG2 cell lysate (20 µg/Lane)
Lane 3: HEK-293 cell lysate (20 µg/Lane)
Lane 4: HeLa cell lysate (20 µg/Lane)
Lane 5: A549 cell lysate (20 µg/Lane)
Lane 6: U-87 MG cell lysate (20 µg/Lane)
Lane 7: K-562 cell lysate (16 µg/Lane)

Predicted band size: 125 kDa
Observed band size: 140 kDa

Exposure time: 2 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 (HA721510) 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 1:100,000 dilution was used for 1 hour at room temperature.

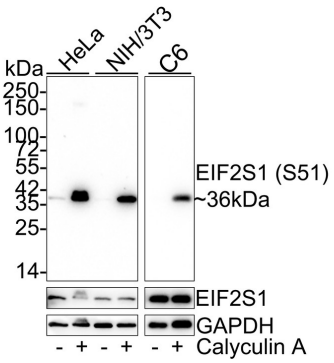

Fig6: Western blot analysis of Phospho-EIF2S1 (S51) on different lysates with Rabbit anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/2,000 dilution.

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.

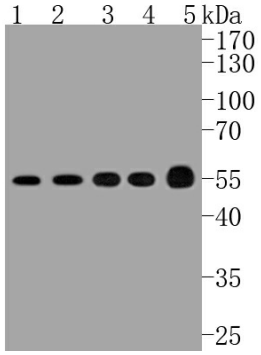

Fig7: Western blot analysis of ATF4 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1612-37, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Hela cell lysate
Lane 2: PC-12 cell lysate
Lane 3: HL-60 cell lysate
Lane 4: K562 cell lysate
Lane 5: human lung carcinoma tissue lysate

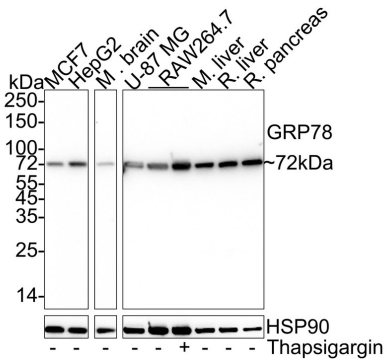

Fig8: Western blot analysis of GRP78 / BIP on different lysates with Mouse anti-GRP78 / BIP antibody (HA601076) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate (15 µg/Lane)
Lane 2: HepG2 cell lysate (15 µg/Lane)
Lane 3: Mouse brain tissue lysate (30 µg/Lane)
Lane 4: U-87 MG cell lysate (30 µg/Lane)
Lane 5: RAW264.7 cell lysate (30 µg/Lane)
Lane 6: RAW264.7 treated with 300nM Thapsigargin for 18 hours cell lysate (30 µg/Lane)
Lane 7: Mouse liver tissue lysate (30 µg/Lane)
Lane 8: Rat liver tissue lysate (30 µg/Lane)
Lane 9: Rat pancreas tissue lysate (30 µg/Lane)

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

Exposure time: Lane 1-3: 11 seconds; Lane 4-9: 4 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 (HA601076) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

Background References

1. Ferro-Novick S, Reggiori F, Brodsky JL. ER-Phagy, ER Homeostasis, and ER Quality Control: Implications for Disease. Trends Biochem Sci. 2021 Aug;46(8):630-639.
2. Sano R, Reed JC. ER stress-induced cell death mechanisms. Biochim Biophys Acta. 2013 Dec;1833(12):3460-3470.
3. Hübner CA, Dikic I. ER-phagy and human diseases. Cell Death Differ. 2020 Mar;27(3):833-842.