

Ferroptosis Antibody Sampler Kit

HAK21028



Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
GPX4 [ET1706-45]	20μl	WB, IHC-P, IF-Cell, FC	H, M, R, Z	22 kDa
NCOA4 [HA722283]	20μl	IHC-P, IF-Tissue	H, M, R	70 kDa
Keap1 [HA721525]	20μl	WB, IHC-P, FC	H, M, R	70 kDa
Nrf2 [HA721432]	20μl	WB, IF-Cell	H, M	68 kDa
CD98 [HA601124]	20μl	WB, IHC-P	H	kDa
xCT / SLC7A11 [HA721868]	20μl	WB, IP	H, M, R	55 kDa
DMT1 [HA723078]	20μl	WB, IHC-P, FC	H	62 kDa
Ferritin Heavy Chain [ET1610-78]	20μl	WB, IF-Cell, IF-Tissue, IHC-P, FC, IHC-Fr	H, M, Z, R	21 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 Ferroptosis Antibody Sampler Kit provides an economical means of detecting proteins involved in ferroptosis. The 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°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

Background Ferroptosis is an iron-dependent form of regulated cell death associated with an increase in lipid peroxides. Ferroptosis is regulated by signaling pathways that control iron storage and oxidative stress. Iron homeostasis is controlled, in part, by ferritin, an iron storage protein consisting of a complex of heavy (FTH1) and light (FTL) chains. Levels of ferritin may be regulated by a selective autophagy process targeting ferritin, termed ferritinophagy.

This pathway is mediated by ARA70, a selective cargo receptor for ferritin . The divalent metal transporter SLC11A2/DMT1/NRAMP2 regulates iron homeostasis through non-heme absorption in the intestine .

GPX4 converts GSH into oxidized glutathione (GSSH) and reduces cytotoxic lipid peroxides. The glutathione peroxidase pathway is further regulated by System Xc-, an amino acid antiporter consisting of a disulfide-linked heterodimer of SLC7A11/xCT and SLC3A2/4F2hc/CD98, and is inhibited by the ferroptosis inducer erastin . Regulation of genes involved in oxidative stress, including GPX4, are largely controlled by the transcription factor NRF2 and serves as a defense against ferroptosis .

Under normal conditions, expression of NRF2 is inhibited through interaction with KEAP1, part of a ubiquitin E3 ligase complex that leads to NRF2 proteasomal degradation. Oxidative stress leads to conformational changes in KEAP1 that disrupts this interaction, resulting in stabilization of NRF2.

Database links: UniProt ID: P36969, O70325, P36970, Q13772, 27057, 619385, Q14145, Q9Z2X8, P57790, Q16236, Q60795, P08195, Q9UPY5, Q9WTR6, 310392, P49281, P02794, P09528,

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

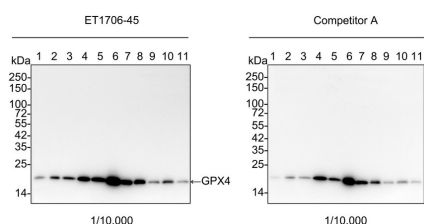
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Western blot analysis of GPX4 on different lysates with Rabbit anti-GPX4 antibody (ET1706-45) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution.



Lane 1: HeLa cell lysate (20 µg/Lane)
 Lane 2: A549 cell lysate (20 µg/Lane)
 Lane 3: HepG2 cell lysate (20 µg/Lane)
 Lane 4: U-937 cell lysate (20 µg/Lane)
 Lane 5: HEK-293 cell lysate (20 µg/Lane)
 Lane 6: Mouse testis tissue lysate (20 µg/Lane)
 Lane 7: Rat testis tissue lysate (20 µg/Lane)
 Lane 8: Mouse kidney tissue lysate (20 µg/Lane)
 Lane 9: Rat kidney tissue lysate (20 µg/Lane)
 Lane 10: Mouse brain tissue lysate (20 µg/Lane)
 Lane 11: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 22 kDa
 Observed band size: 20 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 (ET1706-45) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution were used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

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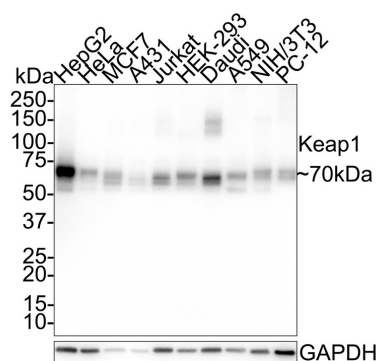


Fig2: Western blot analysis of Keap1 on different lysates with Rabbit anti-Keap1 antibody (HA721525) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate
 Lane 2: HeLa cell lysate
 Lane 3: MCF7 cell lysate
 Lane 4: A431 cell lysate
 Lane 5: Jurkat cell lysate
 Lane 6: HEK-293 cell lysate
 Lane 7: Daudi cell lysate
 Lane 8: A549 cell lysate
 Lane 9: NIH/3T3 cell lysate
 Lane 10: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 70 kDa

Observed band size: 70 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 (HA721525) 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.

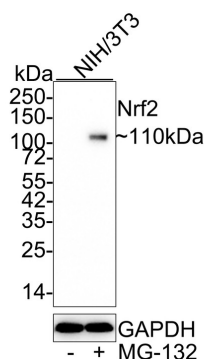


Fig3: Western blot analysis of Nrf2 on different lysates with Rabbit anti-Nrf2 antibody (HA721432) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate

Lane 2: NIH/3T3 treated with 10 μ M MG-132 for 8 hours cell lysate

Lysates/proteins at 20 μ g/Lane.

Predicted band size: 68 kDa

Observed band size: 110 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 (HA721432) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

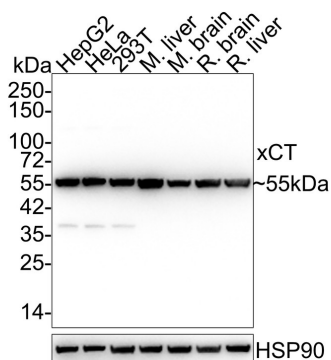


Fig4: Western blot analysis of xCT on different lysates with Rabbit anti-xCT antibody (HA721868) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate (20 μ g/Lane)

Lane 2: HeLa cell lysate (20 μ g/Lane)

Lane 3: 293T cell lysate (20 μ g/Lane)

Lane 4: Mouse liver tissue lysate (40 μ g/Lane)

Lane 5: Mouse brain tissue lysate (40 μ g/Lane)

Lane 6: Rat brain tissue lysate (40 μ g/Lane)

Lane 7: Rat liver tissue lysate (40 μ g/Lane)

Predicted band size: 55 kDa

Observed band size: 55 kDa

Exposure time: 5 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 (HA721868) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 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. Cao JY, Dixon SJ. Mechanisms of ferroptosis. *Cell Mol Life Sci.* 2016 Jun;73(11-12):2195-209. doi: 10.1007/s00018-016-2194-1. Epub 2016 Apr 5.
2. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R, Tang D. Ferroptosis: process and function. *Cell Death Differ.* 2016 Mar;23(3):369-79.
3. Fan Z, Wirth AK, Chen D, Wruck CJ, Rauh M, Buchfelder M, Savaskan N. Nrf2-Keap1 pathway promotes cell proliferation and diminishes ferroptosis. *Oncogenesis.* 2017 Aug 14;6(8):e371.
4. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, Brown LM, Girotti AW, Cornish VW, Schreiber SL, Stockwell BR. Regulation of ferroptotic cancer cell death by GPX4. *Cell.* 2014 Jan 16;156(1-2):317-331.
5. Dixon SJ, Patel DN, Welsch M, Skouta R, Lee ED, Hayano M, Thomas AG, Gleason CE, Tatonetti NP, Slusher BS, Stockwell BR. Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. *Elife.* 2014 May 20;3:e02523.

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