Anti-Keap1 Antibody [PSH0-90]

HA721525

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 70 kDa
Clone number:	PSH0-90

Description: Kelch-like ECH-associated protein 1 is a protein that in humans is encoded by the Keap1 gene. Keap1 has four discrete protein domains. The N-terminal Broad complex, Tramtrack and Bric-à-Brac (BTB) domain contains the Cys151 residue, which is one of the important cysteines in stress sensing. The intervening region (IVR) domain contains two critical cysteine residues, Cys273 and Cys288, which are a second group of cysteines important for stress sensing. A double glycine repeat (DGR) and C-terminal region (CTR) domains collaborate to form a β-propeller structure, which is where Keap1 interacts with Nrf2. Mutations in KEAP1 that result in loss-of-function are not linked to familial cancers, though they do predispose individuals to multinodular goiters. The proposed mechanism leading to goiter formation is that the redox stress experienced when the thyroid produces hormones selects for loss of heterozygosity of KEAP1, leading to the goiters.

Immunogen: Recombinant protein within human Keap1 aa 275-624 / 624.

Positive control: HepG2 cell lysate, HeLa cell lysate, MCF7 cell lysate, A431 cell lysate, Jurkat cell lysate, HEK-293 cell lysate, Daudi cell lysate, A549 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, mouse kidney tissue, rat kidney tissue, PC-12.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q14145 Human | Q9Z2X8 Mouse | P57790 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
FC	1:500-1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

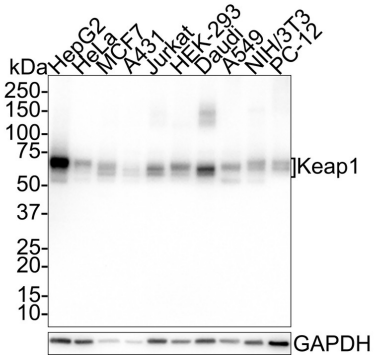

Fig1: Western blot analysis of Keap1 on different lysates with Rabbit anti-Keap1 antibody (HA721525) at 1/1,000 dilution.

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: 55-70 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.

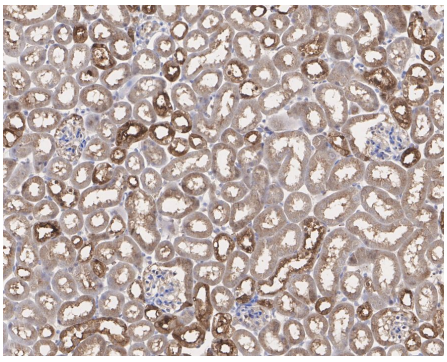

Fig2: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Keap1 antibody (HA721525) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA721525) 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.

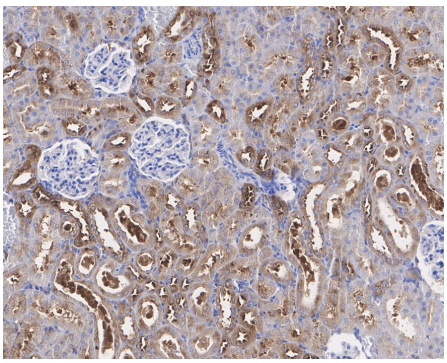

Fig3: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Keap1 antibody (HA721525) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA721525) 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.

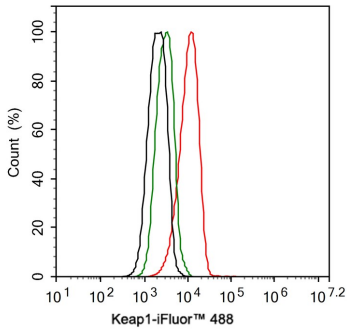

Fig4: Flow cytometric analysis of PC-12 cells labeling Keap1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721525, 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).

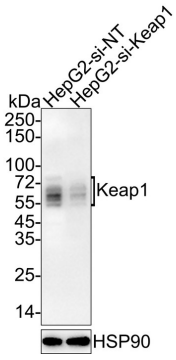

Fig5: Western blot analysis of Keap1 on different lysates with Rabbit anti-Keap1 antibody (HA721525) at 1/1,000 dilution.

Lane 1: HepG2-si NT cell lysate
Lane 2: HepG2-si Keap1 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 1 minute 55 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 (HA721525) at 1/1,000 dilution was used in 5% BSA at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

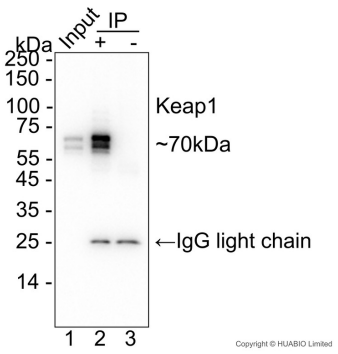

Fig6: Keap1 was immunoprecipitated from 0.2 mg HeLa cell lysate with HA721525 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA721525 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA721525 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA721525 in HeLa cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 12 seconds; ECL: K1801

Background References

1. Baird L et al. The Molecular Mechanisms Regulating the KEAP1-NRF2 Pathway. Mol Cell Biol. 2020 Jun
2. Koppula P et al. A targetable CoQ-FSP1 axis drives ferroptosis- and radiation-resistance in KEAP1 inactive lung cancers. Nat Commun. 2022 Apr