Anti-GPX4 Antibody [JU11-31]

ET1706-45

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 22 kDa
Clone number:	JU11-31

Description: Glutathione peroxidase (GPx) enzymes are generally selenium-containing tetrameric glycoproteins that help prevent lipid peroxidation of cell membranes. GPx enzymes reduce lipid hydroperoxides to alcohols, and reduce free hydrogen peroxide to water. GPx members are among the few proteins known in higher vertebrates to contain selenocysteine, which occurs at the active site of glutathione peroxidase and is coded by the nonsense (stop) codon TGA. There are eight GPx homologs (GPx-1-8). GPx-1, Gpx-2 and Gpx-3 exist as homotetramers. Gpx-4 has a high tendancy to form high molecular weight oligomers.

Immunogen: Synthetic peptide within Human GPX4 aa 23-72 / 197.

Positive control: HeLa cell lysate, A549 cell lysate, HepG2 cell lysate, U-937 cell lysate, HEK-293 cell lysate, mouse testis tissue lysate, rat testis tissue lysate, mouse kidney tissue lysate, rat kidney tissue lysate, mouse brain tissue lysate, rat brain tissue lysate, zebrafish tissue lysates, HEK-293, rat epididymis tissue, human kidney tissue, mouse testis tissue, MCF-7, PC-3M, LOVO.

Subcellular location: Mitochondrion, Cytoplasm.

Database links: SwissProt: P36969 Human | O70325 Mouse | P36970 Rat

Recommended Dilutions:

WB	1:10,000
IHC-P	1:200-1:1,000
IF-Cell	1:50-1:200
FC	1: 10,000

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℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

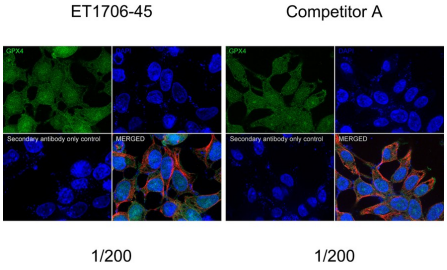

Fig1: Immunocytochemistry analysis of HEK-293 cells labeling GPX4 with Rabbit anti-GPX4 antibody (ET1706-45) at 1/200 dilution and competitor's antibody at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GPX4 antibody (ET1706-45) at 1/200 dilution and competitor's antibody at 1/200 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

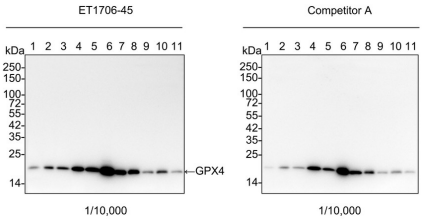

Fig2: Western blot analysis of GPX4 on different lysates with Rabbit anti-GPX4 antibody (ET1706-45) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution.

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℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room

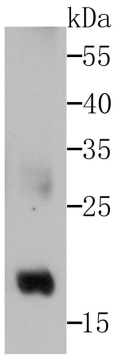

Fig3: Western blot analysis of GPX4 on zebrafish tissue lysates with Rabbit anti-GPX4 antibody (ET1706-45) at 1/500 dilution.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 22 kDa
Observed band size: 17 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 (ET1706-45) 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 1:5,000 dilution was used for 1 hour at room temperature.

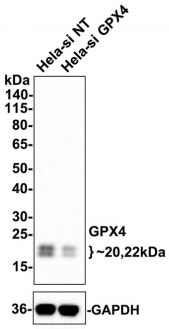

Fig4: Western blot analysis of GPX4 on different lysates with Rabbit anti-GPX4 antibody (ET1706-45) at 1/500 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si GPX4 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 22 kDa
Observed band size: 20,22 kDa

Exposure time: 1 minutes 40 seconds;

4-20% SDS-PAGE gel.

ET1706-45 was shown to specifically react with GPX4 in Hela-si NT cells. Weakened band was observed when Hela-si GPX4 sample was tested. Hela-si NT and Hela-si GPX4 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 (ET1706-45, 1/500) and Loading control antibody (Rabbit anti-GAPDH , ET1601-4, 1/10,000) were used in 5% BSA 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.

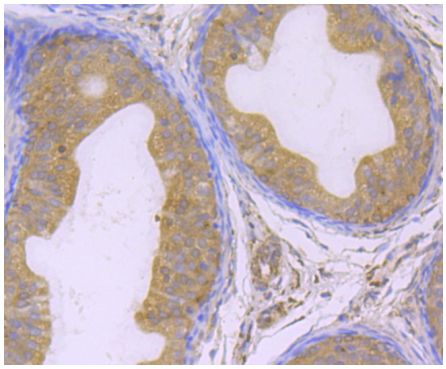

Fig5: Immunohistochemical analysis of paraffin-embedded rat epididymis tissue with Rabbit anti-GPX4 antibody (ET1706-45) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) 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 (ET1706-45) at 1/200 dilution for 0.5 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.

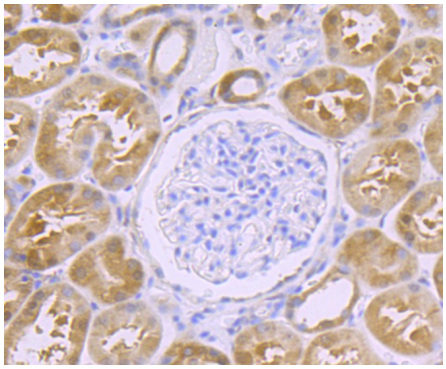

Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-GPX4 antibody (ET1706-45) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) 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 (ET1706-45) at 1/200 dilution for 0.5 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.

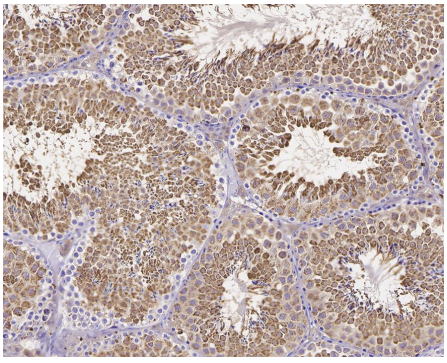

Fig7: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-GPX4 antibody (ET1706-45) at 1/1,000 dilution.

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 (ET1706-45) 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.

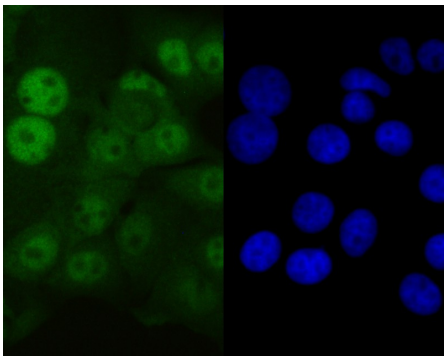

Fig8: Immunocytochemistry analysis of MCF-7 cells labeling GPX4 with Rabbit anti-GPX4 antibody (ET1706-45) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-GPX4 antibody (ET1706-45) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

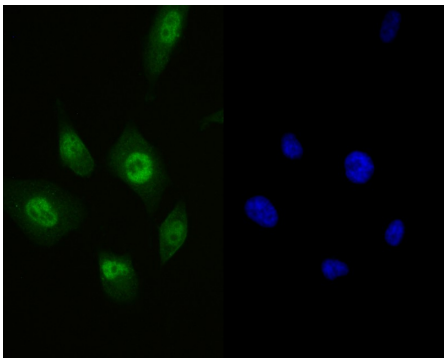

Fig9: Immunocytochemistry analysis of PC-3M cells labeling GPX4 with Rabbit anti-GPX4 antibody (ET1706-45) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-GPX4 antibody (ET1706-45) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

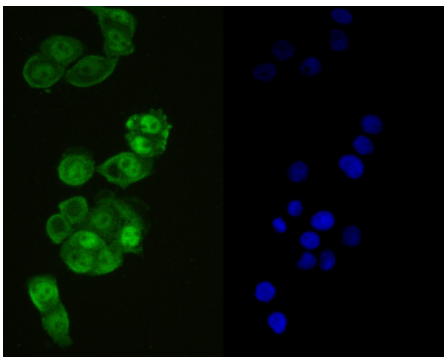

Fig10: Immunocytochemistry analysis of LOVO cells labeling GPX4 with Rabbit anti-GPX4 antibody (ET1706-45) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-GPX4 antibody (ET1706-45) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

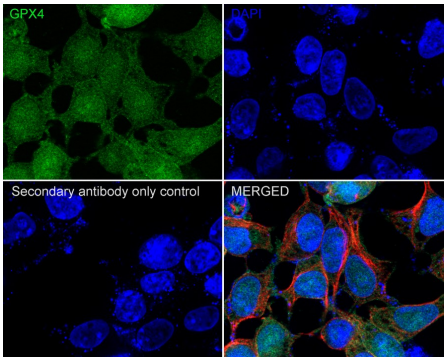

Fig11: Immunocytochemistry analysis of HEK-293 cells labeling GPX4 with Rabbit anti-GPX4 antibody (ET1706-45) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GPX4 antibody (ET1706-45) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

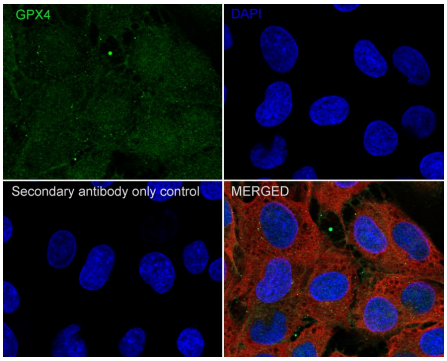

Fig12: Immunocytochemistry analysis of C6 cells labeling GPX4 with Rabbit anti-GPX4 antibody (ET1706-45) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GPX4 antibody (ET1706-45) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

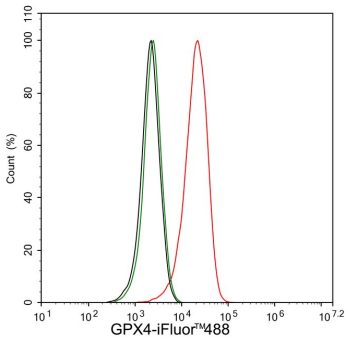

Fig13: Flow cytometric analysis of HEK-293 cells labeling GPX4.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1706-45, 1μg/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).

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

Background References

1. Luna-Sánchez M et al. CoQ deficiency causes disruption of mitochondrial sulfide oxidation, a new pathomechanism associated with this syndrome. EMBO Mol Med 9:78-95 (2017).
2. Kagan VE et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. Nat Chem Biol 13:81-90 (2017).