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Anti-Glutathione Peroxidase 4/GPX4 Antibody Picoband®

Catalog Number: A02059-1

About GPX4

Glutathione peroxidase 4, also known as GPX4, is an enzyme that in humans is encoded by the GPX4 gene. This gene encodes a member of the glutathione peroxidase protein family. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide, organic hydroperoxide, and lipid peroxides by reduced glutathione and functions in the protection of cells against oxidative damage. Human plasma glutathione peroxidase has been shown to be a selenium-containing enzyme and the UGA codon is translated into a selenocysteine. The encoded protein has been identified as a moonlighting protein based on its ability to serve dual functions as a peroxidase as well as a structural protein in mature spermatozoa. Through alternative splicing and transcription initiation, rat produces proteins that localize to the nucleus, mitochondrion, and cytoplasm. In humans, alternative transcription initiation and the cleavage sites of the mitochondrial and nuclear transit peptides need to be experimentally verified. Alternative splicing results in multiple transcript variants.

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Overview

Product Name	Anti-Glutathione Peroxidase 4/GPX4 Antibody Picoband®
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-Glutathione Peroxidase 4/GPX4 Antibody Picoband® catalog # A02059-1. Tested in ELISA, Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, IF, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na2HPO4.
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	P36969

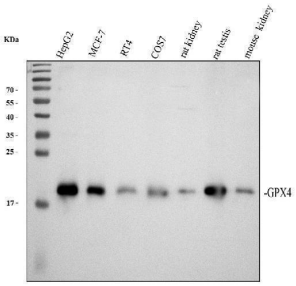
Technical Details

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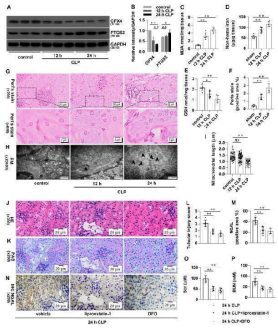
Immunogen	E.coli-derived human Glutathione Peroxidase 4/GPX4 recombinant protein (Position: A30-F197).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.25-0.5 ug/ml, Human, Monkey, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human, Mouse Flow Cytometry(Fixed), 1-3 ug/1x10^6 cells, Human ELISA, 0.1-0.5 ug/ml, - Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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Anti-Glutathione Peroxidase 4/GPX4 Antibody Picoband® (A02059-1) Images



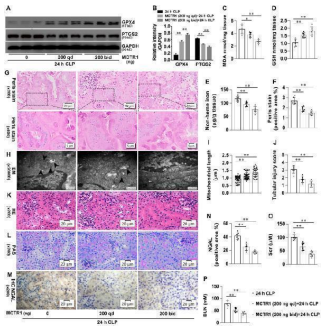
Western blot analysis of Glutathione Peroxidase 4/GPX4 using anti-Glutathione Peroxidase 4/GPX4 antibody (A02059-1). Electrophoresis was performed on a 12% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HepG2 whole cell lysates, Lane 2: human MCF-7 whole cell lysates, Lane 3: human RT4 whole cell lysates, Lane 4: monkey COS-7 whole cell lysates, Lane 5: rat kidney tissue lysates, Lane 6: rat testis tissue lysates, Lane 7: mouse kidney tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-Glutathione Peroxidase 4/GPX4 antigen affinity purified polyclonal antibody (Catalog # A02059-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for Glutathione Peroxidase 4/GPX4 at approximately 19 kDa. The expected band size for Glutathione Peroxidase 4/GPX4 is at 22 kDa.



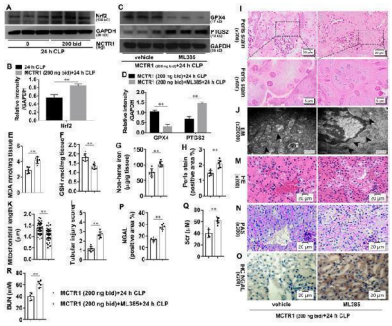
Ferroptosis is present in CLP-induced AKI. Mice were subjected to CLP, then the kidneys were collected at the indicated time. A , B Shown are representative western blotting and quantification of GPX4 and PTGS2. C–E Quantitative analyses of MDA, GSH, and non-heme iron. F Quantitative analyses of Perl’s stain. G Representative Perl’s stain images. H Representative transmission electron microscopy (TEM) images. The black arrow indicates ferroptosis-like mitochondria. I Quantitative analyses of mitochondrial length. Mice were pretreated with liproxstatin-1 (10 mg/kg, ip) or DFO (20 mg/kg, ip) 0.5 h before being subjected to CLP. All kidney samples were collected at 24 h after CLP. J–K Shown are representative Hematoxylin-eosin (HE) stains and Periodic Acid-Schiff (PAS) stain. L Histological analyses of renal tubular injury. M Quantitative analyses of IHC stain of NGAL. N Representative immunohistochemistry (IHC) images for NGAL. O , P Quantitative analyses of Scr and BUN. n = 6 mice/group, mean ± SD were presented. *P < 0.05, **P < 0.01 and ns : P > 0.05 . ip: intraperitoneal Index in PubMed under a CC BY license. PMID: 34961563

Effects of MCTR1 on ferroptosis in CLP-induced AKI. Mice were given MCTR1 once, twice, or not during the 24 h experiment. The once-daily administration mode (qd): Mice were given MCTR1 (200 ng/mice, iv) 0.5 h before being subjected to CLP. The twice-daily administration mode (bid): Mice were given MCTR1 (200 ng/mice, iv) 0.5 h before being

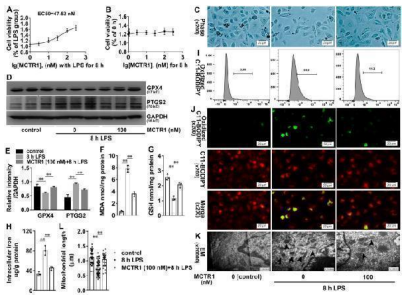
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subjected to CLP, and then an additional MCTR1 (200 ng/mice, iv) was given 12 h after CLP. All kidney samples were collected at 24 h after CLP. A , B Shown are representative western blotting and quantification of GPX4 and PTGS2. C–E Quantitative analyses of MDA, GSH, and non-heme iron. F Quantitative analyses of Perl's stain. G Representative Perl's stain images. H Representative TEM images. The black arrow indicates ferroptosis-like mitochondria. I Quantitative analyses of mitochondrial length. J Histological analyses of renal tubular injury. K , L Shown are representative HE stains and PAS stain. M Representative IHC images for NGAL. N Quantitative analyses of IHC stain of NGAL. O , P Quantitative analyses of serum Scr and BUN. n = 6 mice/group, mean ± SD were presented. **P < 0.01 . iv: intravenous Index in PubMed under a CC BY license. PMID: 34961563



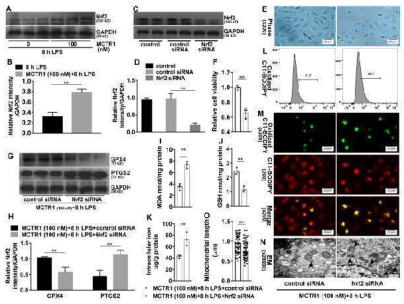
Effects of Nrf2 on MCTR1-regulated ferroptosis in CLP-induced AKI. Mice were given ML385 (30 mg/kg, ip, qd) for 7 d. On day 7, ML385 injection with or without a twice-daily administration mode of MCTR1 as described before in the following experiment. All kidney samples were collected at 24 h after CLP. A–D Shown are representative western blotting and quantification of Nrf2, GPX4, and PTGS2. E – G Quantitative analyses of MDA, GSH, and non-heme iron. H Quantitative analyses of Perl's stain. I Representative Perl's stain images. J Representative TEM images. The black arrow indicates ferroptosis-like mitochondria. K Quantitative analyses of mitochondrial length. L Histological analyses of renal tubular injury. M , N Representative HE stains and PAS stain. O Representative IHC images for NGAL. P Quantitative analyses of IHC stain of NGAL. Q , R Quantitative analyses of serum Scr and BUN. n = 6 mice/group, mean ± SD were presented. **P < 0.01 Index in PubMed under a CC BY license. PMID: 34961563



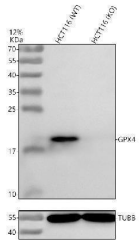
Effects of MCTR1 on LPS-induced lipid peroxidation and ferroptosis in vitro. A Viability curves for HK-2 cells treated with different concentrations (0, 1, 3, 10, 30, 100, and 300 nM) of MCTR1 and LPS (1 ug/ml) for 8 h. B Viability curves for HK-2 cells treated with different concentrations (0, 1, 3, 10, 30, 100, and 300 nM) of MCTR1 for 0 and 8 h. C HK-2 cells treated with MCTR1 (100 nM) and LPS (1 ug/ml) for 8 h, and the visualization of cell viability were evaluated by phase-contrast microscopy. D , E Representative western blotting and quantification of GPX4 and PTGS2 protein. F – H Quantitative analyses of MDA, GSH and, non-heme iron. I Quantitative analyses of oxidized C11-BODIPY 581/591 probe by flow cytometry. J Representative images of C11-BODIPY 581/591 fluorescent ratio-probe. K Representative TEM images. The black arrow indicates ferroptosis-like mitochondria. L Quantitative analyses of mitochondrial length. n = 3, mean ± SD were presented. **P < 0.01 Index in PubMed under a CC BY license. PMID: 34961563

Nrf2 is involved in the inhibitory effects of MCTR1 on LPS-induced ferroptosis. A , B HK-2 cells were treated with

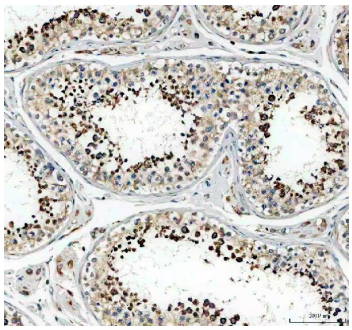
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MCTR1 (100 nM) and LPS (1 ug/ml) for 8 h. Shown are representative western blotting and quantification of Nrf2. HK-2 cells were transfected with 30 nM Nrf2 siRNA for 48 h and then treated with MCTR1 (100 nM) and LPS (1 ug/ml) for 8 h. C , D Representative western blotting and quantification of Nrf2. E Visualization of cell viability were evaluated by phase-contrast microscopy. F Fold change of cell viability. G , H Representative western blotting and quantification of GPX4 and PTGS2. I – K Quantitative analyses of MDA, GSH, and non-heme iron. L Quantitative analyses of oxidized C11-BODIPY 581/591 probe by flow cytometry. M Representative images of C11-BODIPY 581/591 fluorescent ratio-probe. N Representative TEM images. The black arrow indicates ferroptosis-like mitochondria. O Quantitative analyses of mitochondrial length. n = 3, mean ± SD were presented. **P < 0.01 Index in PubMed under a CC BY license. PMID: 34961563



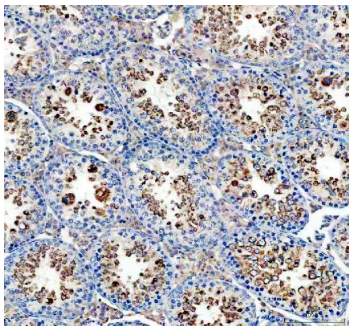
Western blot analysis of Glutathione Peroxidase 4/GPX4 using anti-Glutathione Peroxidase 4/GPX4 antibody (A02059-1). Electrophoresis was performed on a 12% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HCT116- WT whole cell lysates, Lane 2: human HCT116-GPX4 KO whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. Then the membrane was incubated with rabbit anti-Glutathione Peroxidase 4/GPX4 antigen affinity purified polyclonal antibody (A02059-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054) at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for Glutathione Peroxidase 4/GPX4 at approximately 19 kDa. The expected band size for Glutathione Peroxidase 4/GPX4 is at 22 kDa.



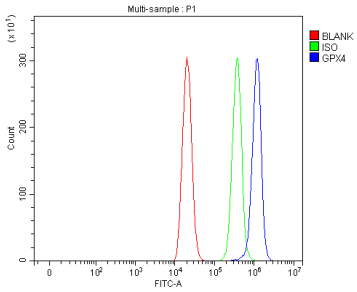
IHC analysis of Glutathione Peroxidase 4/GPX4 using anti-Glutathione Peroxidase 4/GPX4 antibody (A02059-1). Glutathione Peroxidase 4/GPX4 was detected in a paraffin-embedded section of human testis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Glutathione Peroxidase 4/GPX4 Antibody (A02059-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

IHC analysis of Glutathione Peroxidase 4/GPX4 using anti-

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Glutathione Peroxidase 4/GPX4 antibody (A02059-1). Glutathione Peroxidase 4/GPX4 was detected in a paraffin-embedded section of mouse testis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Glutathione Peroxidase 4/GPX4 Antibody (A02059-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



Flow Cytometry analysis of THP-1 cells using anti-Glutathione Peroxidase 4/GPX4 antibody (A02059-1). Overlay histogram showing THP-1 cells stained with A02059-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Glutathione Peroxidase 4/GPX4 Antibody (A02059-1, 1 ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

2 Publications Citing This Product

1. PubMed ID: 10.1186/s13578-021-00734-x, Maresin conjugates in tissue regeneration-1 suppresses ferroptosis in septic acute kidney injury

2. PubMed ID: 10.7150/ijbs.6.316, Cloning, characterization, and expression analysis of goat (Capra hircus) phospholipid hydroperoxide glutathione peroxidase (PHGPx)

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