abcam

Product datasheet

Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free ab219592

KO VALIDATED Recombinant RabMAb

4 References 9 Images

Overview

Product name	Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free
Description	Rabbit monoclonal [EPNCIR144] to Glutathione Peroxidase 4 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, Jurkat cell lysate; HepG2, A549, 293T whole cell lysate; rat heart lysate, mouse ovary, heart and lung lysate; rat, human and mouse testis tissue lysates. ICC/IF: HEK293 and HeLa cells. IHC-P: Human kidney and stomach tissues. Flow Cyt (intra): HeLa cells.
General notes	ab219592 is the carrier-free version of **ab125066**. This antibody was developed as part of a collaboration between Epitomics, the National Cancer Institute's Center for Cancer Research and the lab of Dolph Hatfield. **View antibodies from NCI Center for Cancer Research Collaboration**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply

- Animal-free production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPNCIR144
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab219592 in the following tested applications.

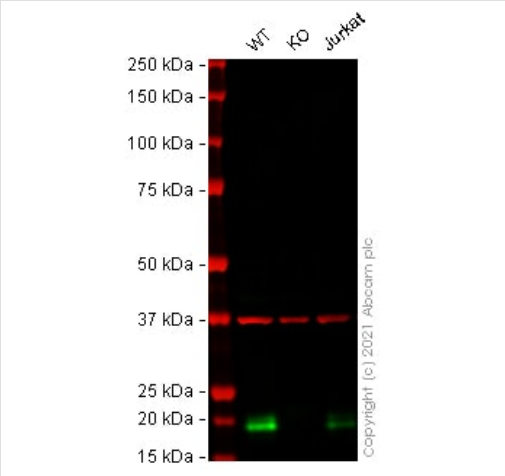
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
WB		Use at an assay dependent concentration. Detects a band of approximately 17 kDa (predicted molecular weight: 22 kDa).

Target

Function	Protects cells against membrane lipid peroxidation and cell death. Required for normal sperm development and male fertility. Could play a major role in protecting mammals from the toxicity of ingested lipid hydroperoxides. Essential for embryonic development. Protects from radiation and oxidative damage.
Tissue specificity	Present primarily in testis.
Sequence similarities	Belongs to the glutathione peroxidase family.
Cellular localization	Mitochondrion. Cytoplasm.

Images



Western blot - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

All lanes : Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (**ab125066**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : GPX4 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

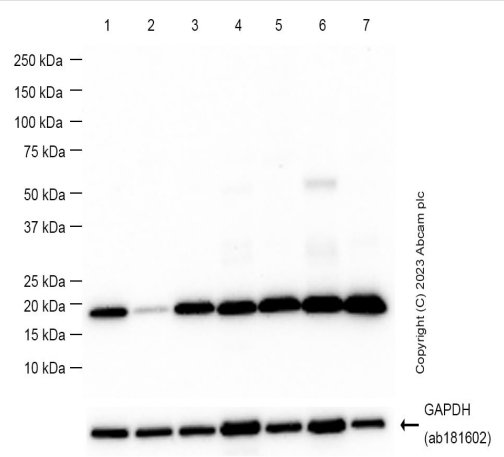
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab125066**).

Lanes 1 - 3: Merged signal (red and green). Green - **ab125066** observed at 20 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab125066 was shown to react with Glutathione Peroxidase 4 in wild-type HeLa cells in Western blot with loss of signal observed in GPX4 knockout cell line **ab262509** (knockout cell lysate **ab263935**). Wild-type HeLa and GPX4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab125066** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

All lanes : Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (**ab125066**) at 1/1000 dilution

Lane 1 : 293T (Human embryonic kidney epithelial cell) whole cell lysate
Lane 2 : A549 (Human lung carcinoma epithelial cell) whole cell lysate
Lane 3 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate
Lane 4 : Rat heart lysate
Lane 5 : Mouse ovary lysate
Lane 6 : Mouse heart lysate
Lane 7 : Mouse lung lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

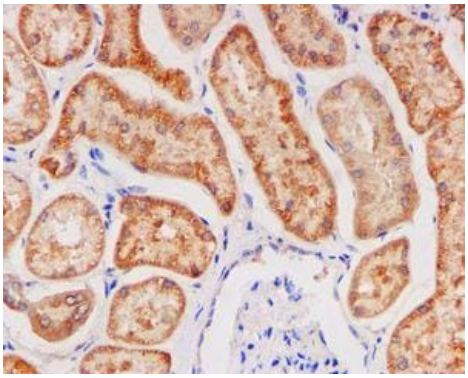
Predicted band size: 22 kDa
Observed band size: 22 kDa

Exposure time: 20 seconds

This data was developed using the same antibody clone in a different buffer formulation (**ab125066**).

Blocking/Diluting buffer and concentration 5% NFDM/TBST.

ab181602 was used as GAPDH loading control.

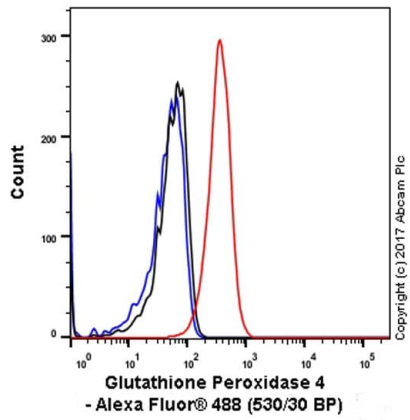


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

Unpurified **ab125066**, at a 1/100 dilution, staining Glutathione Peroxidase 4 in paraffin-embedded Human kidney tissue by immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab125066**).

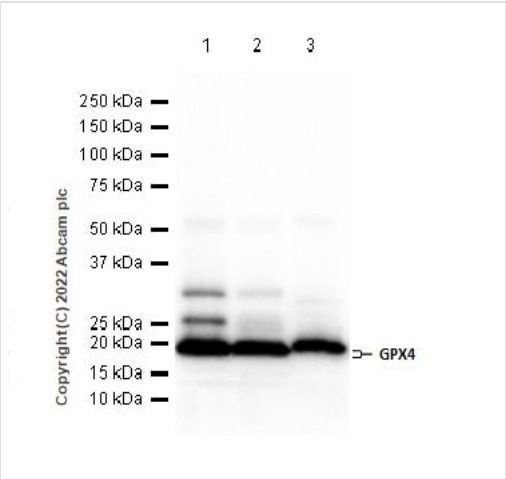
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Glutathione Peroxidase 4 (red) with **ab125066** at a 1/400 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (**ab172730**). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab125066**).



Western blot - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

All lanes : Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (**ab125066**) at 1/1000 dilution

Lane 1 : Rat testis tissue lysate
Lane 2 : Mouse testis tissue lysate
Lane 3 : Human testis tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

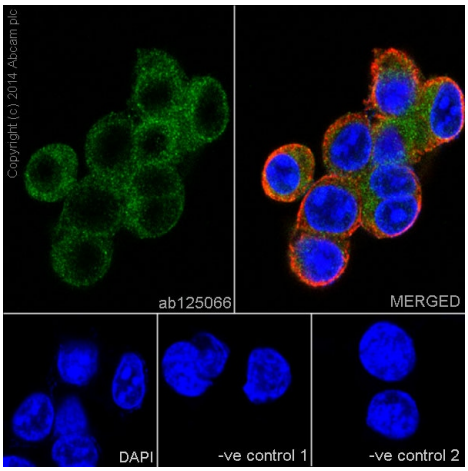
Predicted band size: 22 kDa
Observed band size: 19,22 kDa

Exposure time: 1 second

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

This antibody can detect 19kda cytoplasmic form and 22kda mitochondrial isoform.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab125066**).

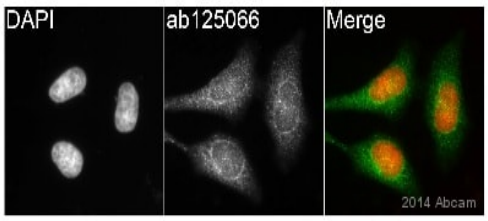


Immunocytochemistry/ Immunofluorescence - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

Immunofluorescence staining of HEK293 cells with purified **ab125066** at a working dilution of 1/200, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified **ab125066** was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (**ab125066**).

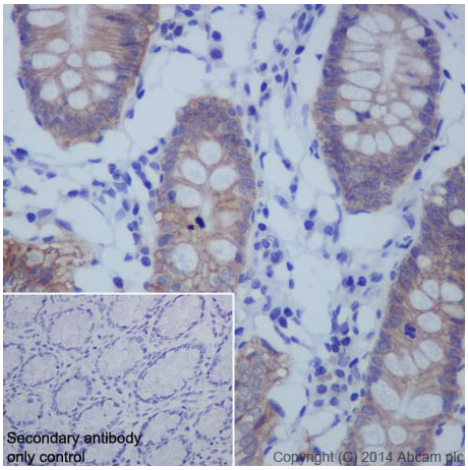


Immunocytochemistry/ Immunofluorescence - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

This image is courtesy of an Abreview submitted by Kirk McManus.

Unpurified **ab125066** staining Glutathione Peroxidase 4 in the HeLa cell line from Human cervical cancer by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with methanol. Samples were incubated with primary antibody (1/500 in PBS) for 1 hour at 22°C. **ab150081** an Alexa Fluor®488-conjugated Goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody. Nuclear staining was carried out with DAPI.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab125066**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

Immunohistochemical staining of paraffin embedded human stomach with purified **ab125066** at a working dilution of 1/50. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab125066**).

Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] - BSA and Azide free (ab219592)

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