

Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] ab125066

敲除验证
重组
RabMAb

★★★★★
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概述

产品名称	Anti-Glutathione Peroxidase 4抗体[EPNCIR144]
描述	兔单克隆抗体[EPNCIR144] to Glutathione Peroxidase 4
宿主	Rabbit
经测试应用	适用于: Flow Cyt (Intra), WB, IHC-P, ICC/IF
种属反应性	与反应: Mouse, Rat, Human
免疫原	Synthetic peptide within Mouse Glutathione Peroxidase 4. The exact sequence is proprietary. Database link: O70325
阳性对照	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.
常规说明	<p>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.</p> <p>Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).</p> <p>See other anti-rabbit secondary antibodies that can be used with this antibody.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.</p>

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	EPNCIR144
同种型	IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab125066于以下的经测试应用

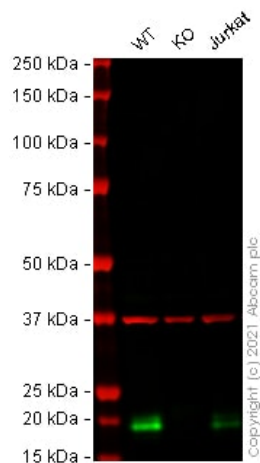
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/400.
WB	★★★★★ (8)	1/1000 - 1/10000. Detects a band of approximately 17 kDa (predicted molecular weight: 22 kDa).
IHC-P	★★★★☆ (2)	1/50 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See <u>IHC antigen retrieval protocols</u> .
ICC/IF	★★★★☆ (2)	Use at an assay dependent concentration.

靶标

功能	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.
组织特异性	Present primarily in testis.
序列相似性	Belongs to the glutathione peroxidase family.
细胞定位	Mitochondrion. Cytoplasm.

图片



Western blot - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (ab125066)

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.

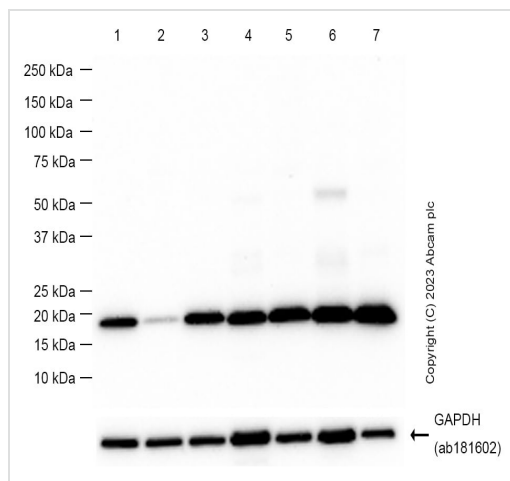
Performed under reducing conditions.

Predicted band size: 22 kDa

Observed band size: 20 kDa

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] (ab125066)

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

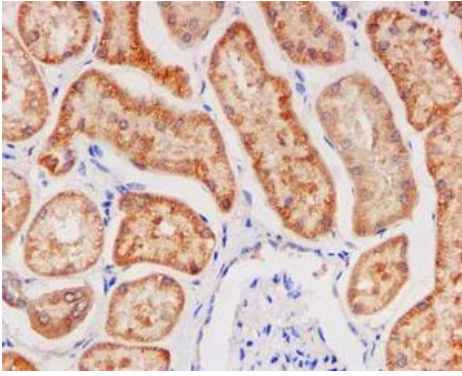
Predicted band size: 22 kDa

Observed band size: 22 kDa

Exposure time: 20 seconds

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

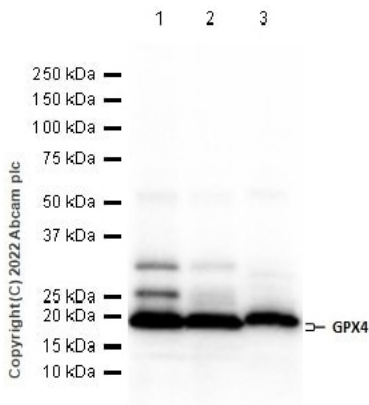
[ab181602](#) was used as GAPDH loading control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (ab125066)

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

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



Western blot - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (ab125066)

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

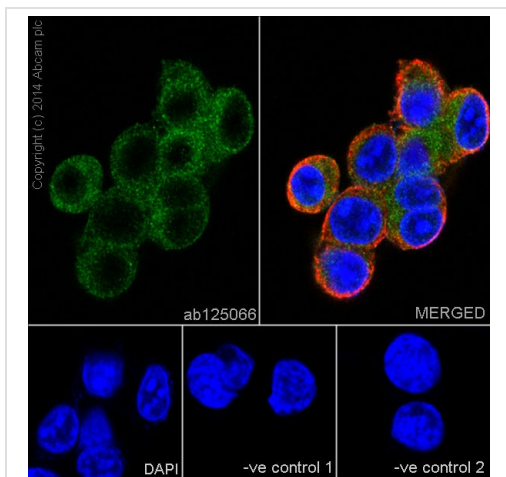
Predicted band size: 22 kDa

Observed band size: 19,22 kDa

Exposure time: 1 second

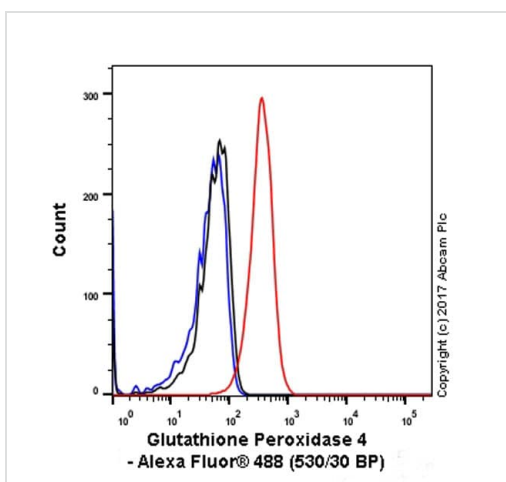
Blocking/Diluting buffer and concentration: 5% NFDM/TBST

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



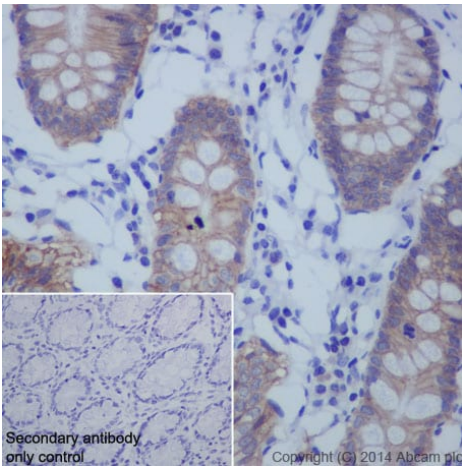
Immunocytochemistry/ Immunofluorescence - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (ab125066)

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.



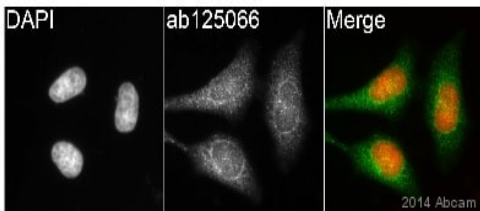
Flow Cytometry (Intracellular) - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (ab125066)

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (ab125066)

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 performed 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.



Immunocytochemistry/ Immunofluorescence - Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (ab125066)

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.

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Anti-Glutathione Peroxidase 4 antibody
[EPNCIR144] (ab125066)

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