abcam

Product datasheet

# Anti-P4HB antibody [RL90] ab2792

48 Abreviews 211 References 17 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-P4HB抗体[RL90] |
| **描述** | **小鼠单克隆抗体**[RL90] to P4HB |
| **宿主** | Mouse |
| **经测试应用** | **适用于:** ELISA, Inhibition Assay, IHC-Fr, IHC-P, WB, IP, Flow Cyt, ICC/IF, Electron Microscopy |
| **种属反应性** | **与反应:** Mouse, Rat, Hamster, Dog, Human, Pig, Monkey, African green monkey<br>**预测可用于:** Drosophila melanogaster |
| **免疫原** | Full length native protein (purified) corresponding to Rat P4HB. Purified rat PDIA1/P4HB protein. Database link: **P04785** |
| **阳性对照** | WB: HeLa, A-431,NIH/3T3, A-375, Hep G2, HL-60, and PC-12. ICC/IF: HeLa, A-431, MCF7, A549 and U2OS cells |
| **常规说明** | The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.<br>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As |

## 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 or -80°C. Avoid freeze / thaw cycle. |
| **存储溶液** | Preservative: 0.05% Sodium azide<br>Constituents: 0.1% BSA, PBS |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |
| **克隆编号** | RL90 |
| **同种型** | IgG2a |

## 应用

**The Abpromise guarantee** Abpromise™承诺保证使用ab2792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 |
|---|---|---|
| **ELISA** | | Use at an assay dependent concentration. |
| **Inhibition Assay** | | Use at an assay dependent concentration. |
| **IHC-Fr** | ★★☆☆☆ (1) | Use at an assay dependent concentration. |
| **IHC-P** | ★★☆☆☆ (2) | 1/100. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol. |
| **WB** | ★★★★★ (15) | 1/1000. Detects a band of approximately 59-61 kDa (predicted molecular weight: 58 kDa). If there is no signal or signal is weak, more concentrated antibody could be used in addition to using less stringent blocking conditions (e.g., BSA instead of milk, incubating the antibody in PBST or TBST only, lower milk percentage). |
| **IP** | | Use at an assay dependent concentration.<br>This antibody has been shown to inhibit the activity of PDI in vitro. It has also been found to inhibit disulfide bond reduction of the HIV protein, gp120, at the cell surface of CHO cells and human lymphoid cells. |
| **Flow Cyt** | | Use 0.5µg for $10^6$ cells.<br>**ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody. |
| **ICC/IF** | ★★★★☆ (26) | Use at an assay dependent concentration. |
| **Electron Microscopy** | | Use at an assay dependent concentration. PubMed: 21886772 |

## 靶标

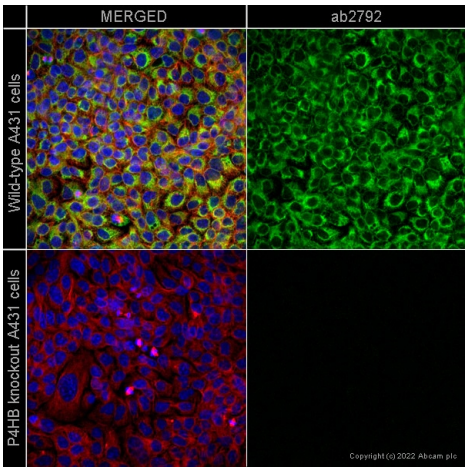
**功能** This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds. At the cell surface, seems to act as a reductase that cleaves disulfide bonds of proteins attached to the cell. May therefore cause structural modifications of exofacial proteins. Inside the cell, seems to form/rearrange disulfide bonds of nascent proteins. At high concentrations, functions as a chaperone that inhibits aggregation of misfolded proteins. At low concentrations, facilitates aggregation (anti-chaperone activity). May be involved with other chaperones in the structural modification of the TG precursor in hormone biogenesis. Also acts a structural subunit of various enzymes such as prolyl 4-hydroxylase and microsomal triacylglycerol transfer protein MTTP.

**序列相似性** Belongs to the protein disulfide isomerase family.
Contains 2 thioredoxin domains.

**细胞定位** Endoplasmic reticulum lumen. Melanosome. Cell membrane. Highly abundant. In some cell types,

seems to be also secreted or associated with the plasma membrane, where it undergoes constant shedding and replacement from intracellular sources (Probable). Localizes near CD4-enriched regions on lymphoid cell surfaces. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

## 图片

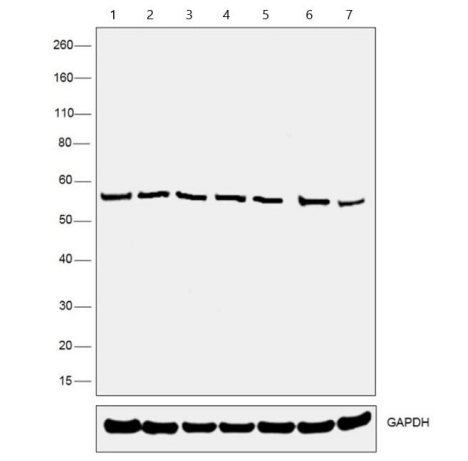



Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [RL90] (ab2792)

ab2792 staining P4HB in wild-type A431 cells (top panel) and P4HB knockout A431 cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab2792 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150084**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.




Western blot - Anti-P4HB antibody [RL90] (ab2792)

**All lanes :** Anti-P4HB antibody [RL90] (ab2792) at 1/2000 dilution

**Lane 1 :** HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
**Lane 2 :** A431 (human epidermoid carcinoma cell line) whole cell lysate
**Lane 3 :** NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate
**Lane 4 :** A-375 (human malignant melanoma cell line) whole cell lysate
**Lane 5 :** HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate
**Lane 6 :** HL-60 (human promyelocytic leukemia cell line) whole cell lysate
**Lane 7 :** PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate

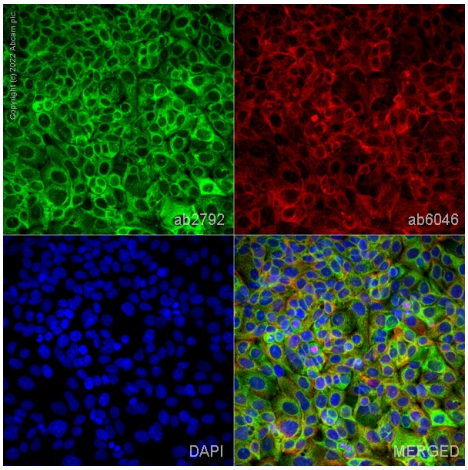
Lysates/proteins at 30 µg per lane.

**Secondary**

**All lanes :** Goat anti-Mouse IgG H+L (HRP) at 1/4000 dilution

**Predicted band size:** 58 kDa

**Observed band size:** 57 kDa

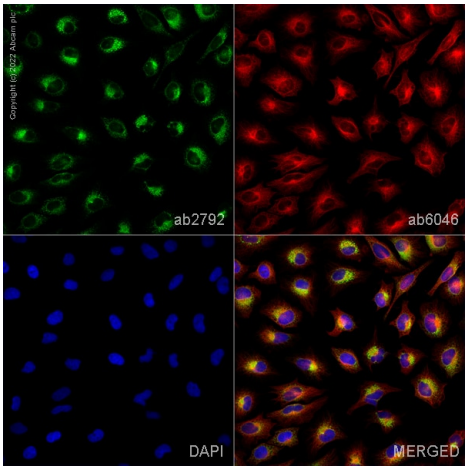



Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [RL90] (ab2792)

ab2792 staining P4HB in MCF7 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab2792 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150084**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

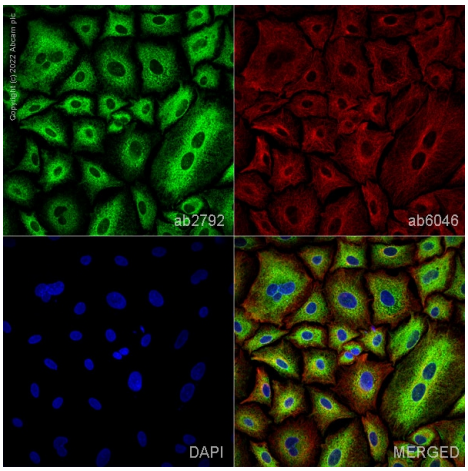



Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [RL90] (ab2792)

ab2792 staining P4HB in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab2792 at 0.2µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150084**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

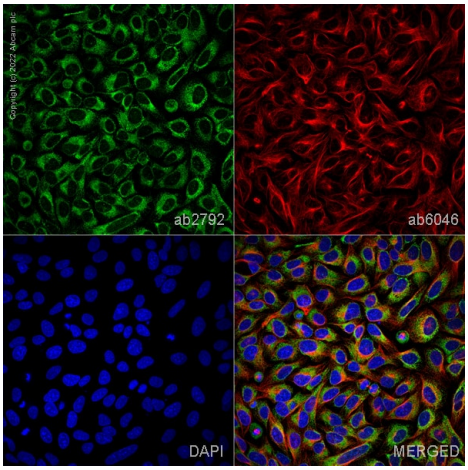



Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [RL90] (ab2792)

ab2792 staining P4HB in A549 cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab2792 at 0.2µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150084**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

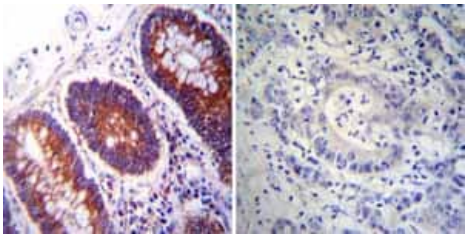



Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [RL90] (ab2792)

ab2792 staining P4HB in U2OS cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab2792 at 0.2µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150084**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

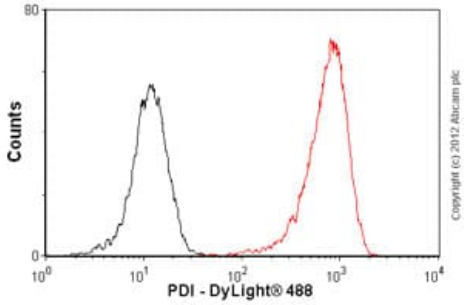
Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



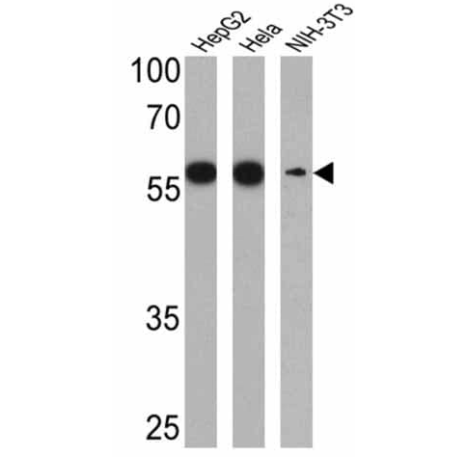
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-P4HB antibody [RL90] (ab2792)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human colon tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing P4HB (PDIA1) ab2792 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



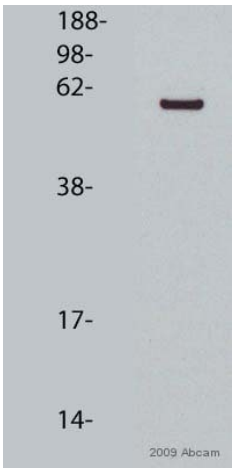

Flow Cytometry - Anti-P4HB antibody [RL90] (ab2792)

Overlay histogram showing HeLa cells stained with ab2792 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab2792, 0.5µg/1x$10^6$ cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (**ab91361**, 1µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.




Western blot - Anti-P4HB antibody [RL90] (ab2792)

Western blot analysis of P4HB (PDIA1) was performed by loading 25 ug of HepG2 (Lane 1) Hela (Lane 2) and NIH-3T3 (Lane 3) cell lysates onto an SDS polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked at 4°C overnight. The membrane was probed with ab2792 at 1:1000 overnight at 4°C and washed in TBST. The membrane was then probed with a HRP-conjugated secondary antibody for 1 hr at room temperature in the dark. Chemiluminescent detection was performed using a ECL Plus Western Blotting Substrate. Results show a band at approx. 57 kDa.




Western blot - Anti-P4HB antibody [RL90] (ab2792)

This image is courtesy of an anonymous Abreview

Anti-P4HB antibody [RL90] (ab2792) at 1/2000 dilution

**Secondary**

Donkey anti mouse IgG2a at 1/10000 dilution

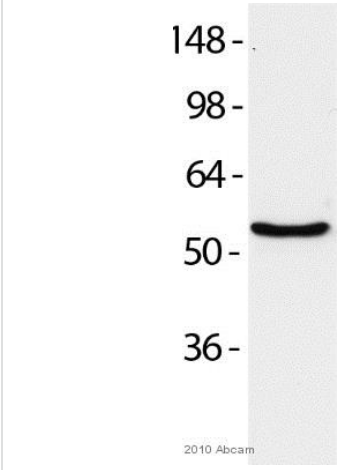
Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 58 kDa

**Observed band size:** 57 kDa

**Exposure time:** 20 seconds




Western blot - Anti-P4HB antibody [RL90] (ab2792)

Anti-P4HB antibody [RL90] (ab2792) at 1/1000 dilution + HT1080 whole cell lysate at 20000 cells

**Secondary**

HRP-conjugated sheep anti-mouse IgG at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 58 kDa

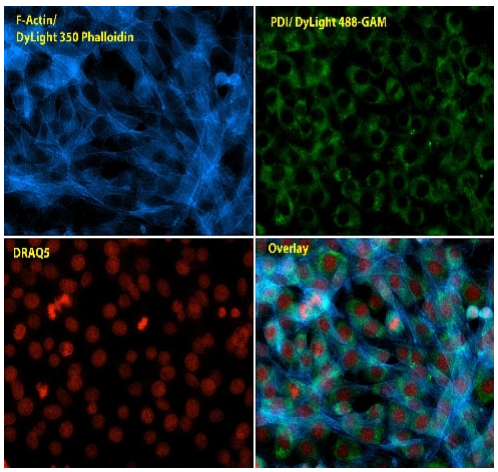
**Observed band size:** 57 kDa

**Exposure time:** 20 seconds

10% SDS-PAGE.

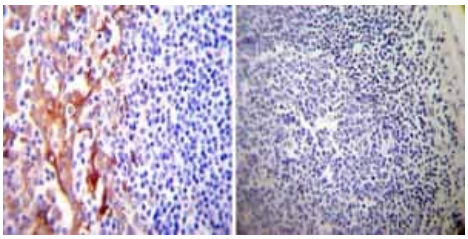
Blocked with 5% milk for 1 hour at 22°C.

Incubated with the primary for 16 hours at 4°C in PBS + 2.5% milk + 0.05% Tween20.



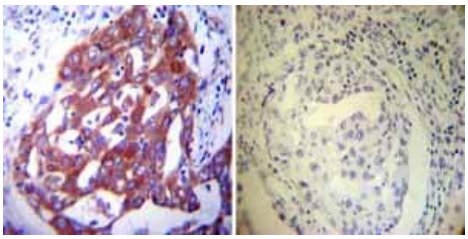

Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [RL90] (ab2792)

Immunocytochemistry/Immunofluorescence analysis of P4HB (PDIA1) (green) in NIH 3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes at room temperature and blocked with 2% BSA in PBS + 0.1% Triton X-100 for 30 minutes at room temperature. Cells were probed with ab2792 (1:75) for at least 1 hour at room temperature and incubated with Dylight 488 goat anti-mouse IgG secondary antibody (1:250) for 30 minutes at room temperature. Actin was stained with Dylight 350 Phalloidin at a dilution of 1:120 (2.5units/ml final concentration) and nuclei (red) were stained with DRAQ5 at a concentration of 1ug/ml for 30 minutes. Images were taken at 20X magnification.



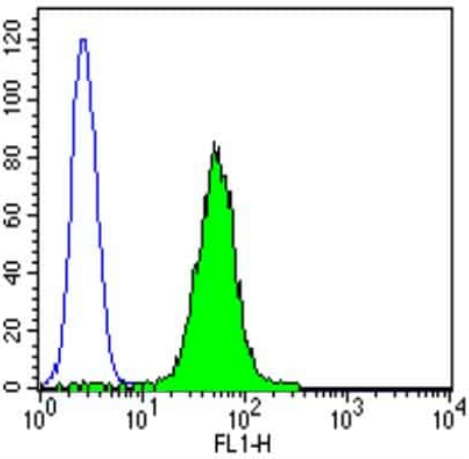
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-P4HB antibody [RL90] (ab2792)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human tonsil tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing P4HB (PDIA1) ab2792 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



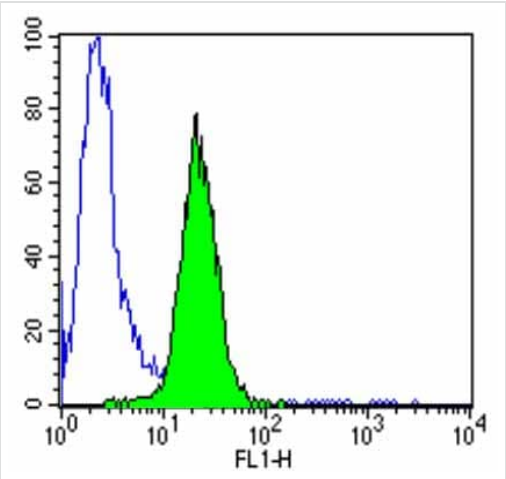
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-P4HB antibody [RL90] (ab2792)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human lung adenocarcinoma tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing P4HB (PDIA1) ab2792 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



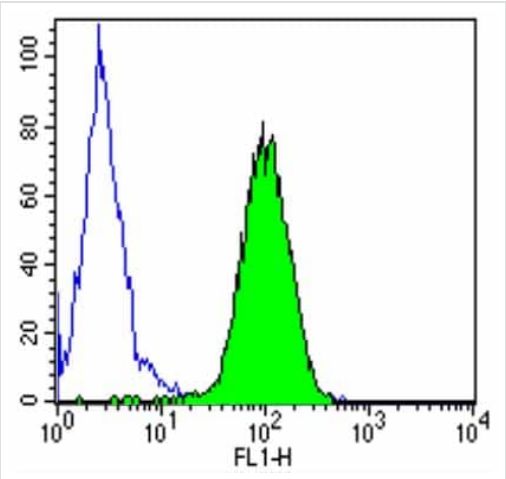

Flow Cytometry - Anti-P4HB antibody [RL90] (ab2792)

Flow cytometry analysis of P4HB (PDIA1) showing positive staining in the membrane and cytoplasm of K562 cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2792 at 1 ug/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.




Flow Cytometry - Anti-P4HB antibody [RL90] (ab2792)

Flow cytometry analysis of P4HB (PDIA1) showing positive staining in the membrane and cytoplasm of NIH/3T3 cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2792 at 0.5 ug/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.




Flow Cytometry - Anti-P4HB antibody [RL90] (ab2792)

Flow cytometry analysis of P4HB (PDIA1) showing positive staining in the membrane and cytoplasm of Hela cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2792 at 1 ug/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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