

Product datasheet

Anti-P4HB antibody [EPR9499] ab137110

KO VALIDATED Recombinant RabMAB

★★★★☆ **6 Abreviews** **14 References** **18 Images**

Overview

Product name	Anti-P4HB antibody [EPR9499]
Description	Rabbit monoclonal [EPR9499] to P4HB
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human P4HB aa 450 to the C-terminus (C terminal). The exact sequence is proprietary.
Positive control	WB: HeLa, HepG2, 293T, Raw264.7, PC-12 and A431 cell lysates. ICC/IF: HeLa, A-431, MCF7, A549 and U2OS cells IHC-P: Human breast carcinoma, human brain, human kidney, mouse liver and rat liver tissues. Flow Cyt (intra): HepG2 and HeLa cells.
General notes	<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>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR9499

Isotype

IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab137110 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)		1/100 - 1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (3)	1/1000 - 1/10000. Predicted molecular weight: 57 kDa.
IHC-P		1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols . For unpurified use at 1/100 - 1/250.
ICC/IF	★★★★★ (3)	1/150 - 1/500.

Target

Function

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.

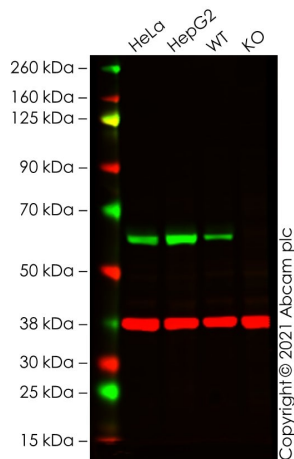
Sequence similarities

Belongs to the protein disulfide isomerase family.
Contains 2 thioredoxin domains.

Cellular localization

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.

Images



Western blot - Anti-P4HB antibody [EPR9499] (ab137110)

All lanes : Anti-P4HB antibody [EPR9499] (ab137110) at 1/1000 dilution

Lane 1 : HeLa cell lysate

Lane 2 : HepG2 cell lysate

Lane 3 : Wild-type A431 cell lysate

Lane 4 : P4HB knockout A431 cell lysate

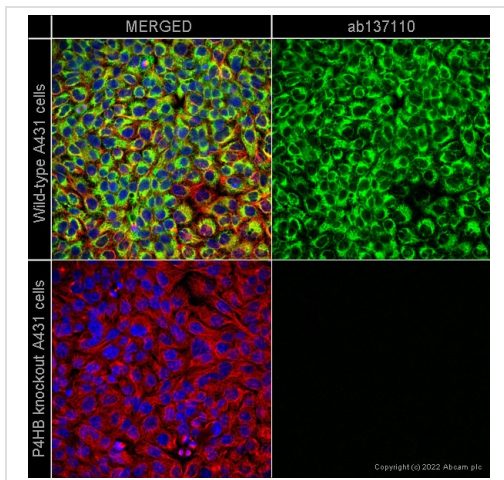
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 57 kDa

Observed band size: 60 kDa

False colour image of Western blot: Anti-P4HB antibody [EPR9499] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] ([ab8245](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab137110 was shown to bind specifically to P4HB. A band was observed at 60 kDa in wild-type HeLa cell lysates with no signal observed at this size in P4HB knockout cell line [ab261887](#) (knockout cell lysate [ab261696](#)). To generate this image, wild-type and P4HB knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1 % Tween[®] 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.

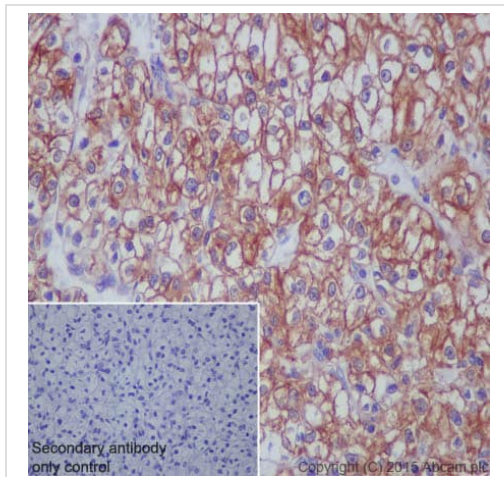


Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [EPR9499] (ab137110)

ab137110 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 ab137110 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

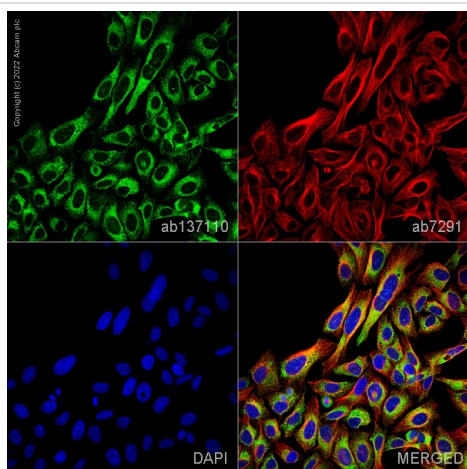
Also suitable in cells fixed with 4% paraformaldehyde (10 min).

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 [EPR9499] (ab137110)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney carcinoma tissue labelling P4HB with purified ab137110 at a dilution of 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

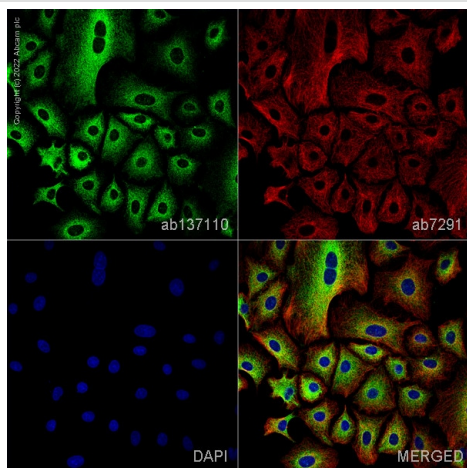


Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [EPR9499] (ab137110)

ab137110 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 ab137110 at 0.2µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed 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.

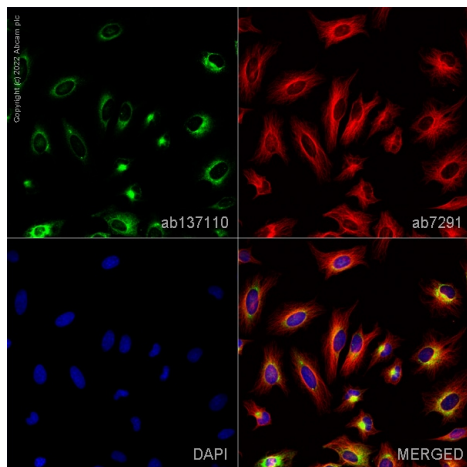


Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [EPR9499] (ab137110)

ab137110 staining P4HB in A549 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 ab137110 at 0.2µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed 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.

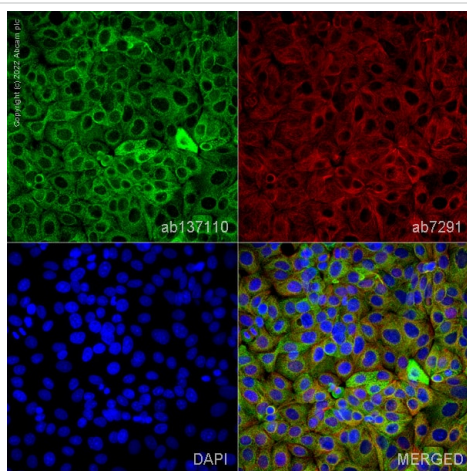


Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [EPR9499] (ab137110)

ab137110 staining P4HB in HeLa 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 ab137110 at 0.2µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed 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.

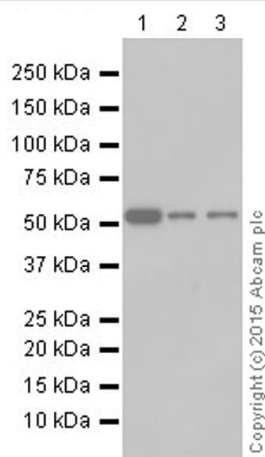


Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [EPR9499] (ab137110)

ab137110 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 ab137110 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed 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 [EPR9499] (ab137110)

All lanes : Anti-P4HB antibody [EPR9499] (ab137110) at 1/10000 dilution (purified)

Lane 1 : HepG2 whole cell lysate

Lane 2 : HeLa whole cell lysate

Lane 3 : HEK293 whole cell lysate

Lysates/proteins at 10 µg per lane.

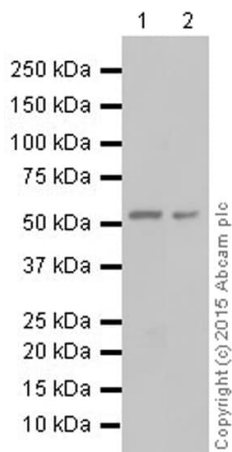
Secondary

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

Predicted band size: 57 kDa

Observed band size: 57 kDa

Blocking and dilution buffer: 5% NFDm/TBST



Western blot - Anti-P4HB antibody [EPR9499] (ab137110)

All lanes : Anti-P4HB antibody [EPR9499] (ab137110) at 1/10000 dilution (purified)

Lane 1 : Raw264.7 whole cell lysate

Lane 2 : PC-12 whole cell lysate

Lysates/proteins at 10 µg per lane.

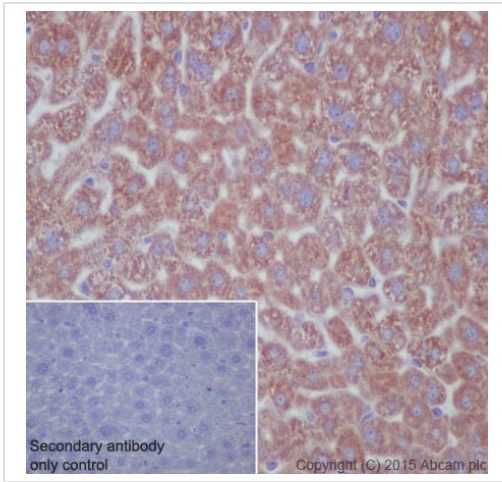
Secondary

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

Predicted band size: 57 kDa

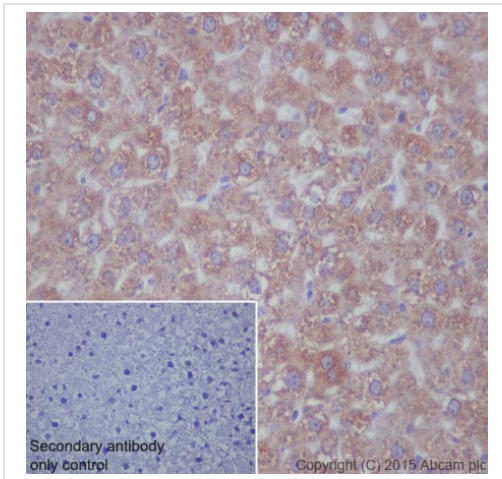
Observed band size: 57 kDa

Blocking and dilution buffer: 5% NFDm/TBST



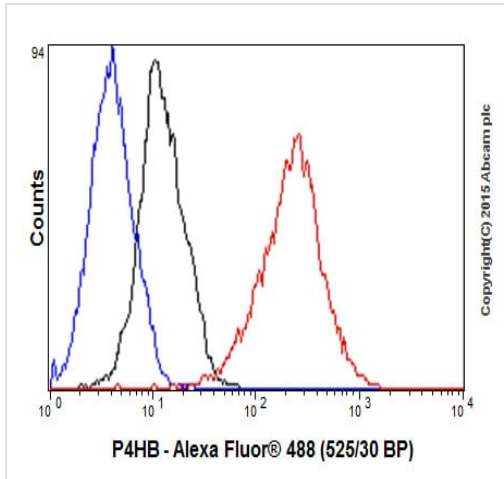
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue labelling P4HB with purified ab137110 at a dilution of 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-P4HB antibody [EPR9499] (ab137110)



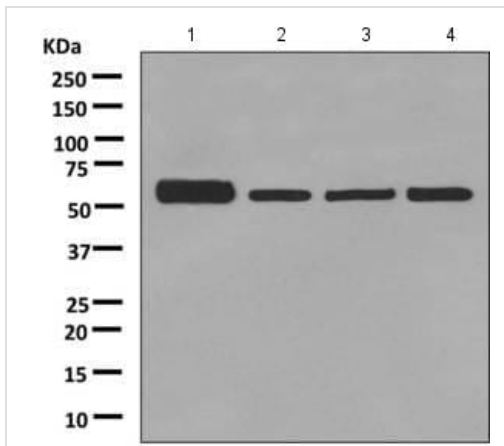
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue labelling P4HB with purified ab137110 at a dilution of 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-P4HB antibody [EPR9499] (ab137110)



Flow Cytometry (Intracellular) - Anti-P4HB antibody [EPR9499] (ab137110)

Intracellular Flow Cytometry analysis of HeLa cells labelling P4HB with purified ab137110 at a dilution of 1/300 (red). Cells were fixed with 4% paraformaldehyde. An Alexa Fluor[®]488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-P4HB antibody [EPR9499] (ab137110)

All lanes : Anti-P4HB antibody [EPR9499] (ab137110) at 1/1000 dilution (unpurified)

Lane 1 : HepG2 cell lysate

Lane 2 : HeLa cell lysate

Lane 3 : 293T cell lysate

Lane 4 : A431 cell lysate

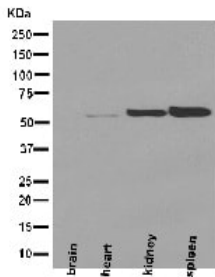
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Developed using the ECL technique.

Predicted band size: 57 kDa



Western blot - Anti-P4HB antibody [EPR9499] (ab137110)

All lanes : Anti-P4HB antibody [EPR9499] (ab137110) at 1/2000 dilution (unpurified)

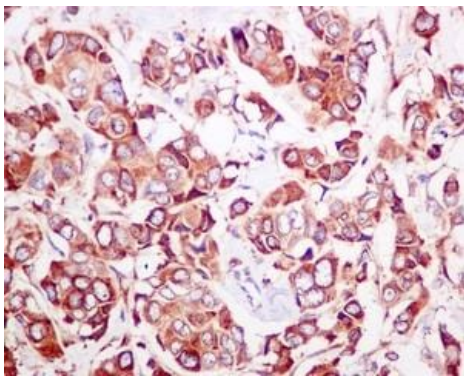
Lane 1 : Mouse brain tissue lysate

Lane 2 : Mouse heart tissue lysate

Lane 3 : Mouse kidney tissue lysate

Lane 4 : Mouse spleen tissue lysate

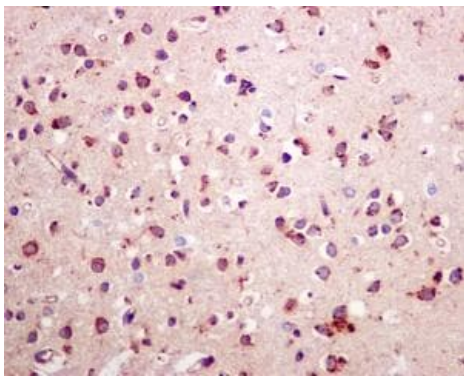
Predicted band size: 57 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-P4HB antibody [EPR9499] (ab137110)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling P4HB with unpurified ab137110 at a dilution of 1/100.

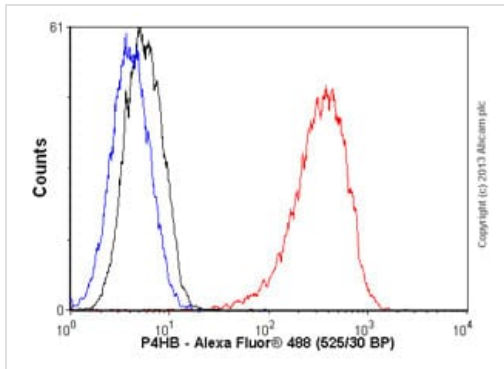
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-P4HB antibody [EPR9499] (ab137110)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human brain tissue labelling P4HB with unpurified ab137110 at a dilution of 1/100.





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



Flow Cytometry (Intracellular) - Anti-P4HB antibody [EPR9499] (ab137110)

Overlay histogram showing HepG2 cells stained with unpurified ab137110 (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 (unpurified ab137110, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit Alexa Fluor® 488 (IgG H&L) ([ab150077](#)) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HepG2 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-P4HB antibody [EPR9499] (ab137110)

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

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