abcam

ab137110

Anti-P4HB antibody [EPR9499]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR9499
Purification technique	Affinity purification Protein A
Concentration	1.461 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Mouse
Dilution info	1/1000
Notes	**For unpurified use at 1/100 - 1/250.** Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/1000
Notes	**For unpurified use at 1/100 - 1/250.** Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Species	Human
Dilution info	1/1000
Notes	**For unpurified use at 1/100 - 1/250.** Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/10000
Notes	-

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/150 - 1/500
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100 - 1/1000
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information P4HB

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 and following phosphorylation by FAM20C, functions as a chaperone that inhibits aggregation of misfolded proteins (PubMed:32149426). 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 as a structural subunit of various enzymes such as prolyl 4-hydroxylase and microsomal triacylglycerol transfer protein MTTP. Receptor for LGALS9; the interaction retains P4HB at the cell surface of Th2 T helper cells, increasing disulfide reductase activity at the plasma membrane, altering the plasma membrane redox state and enhancing cell migration (PubMed:21670307).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	P4HB is a protein known as Protein Disulfide Isomerase (PDI). It plays a significant role in catalyzing the formation of disulfide bonds. This protein helps to fold newly synthesized proteins by shuffling disulfide bonds. It has a molecular mass of around 57 kDa. P4HB is mainly expressed in the endoplasmic reticulum of cells. While its alternative name is ERp59 P4HB is integral to the protein folding machinery inside cells.
Biological function summary	P4HB functions in protein folding and assembly. By acting as a chaperone it protects proteins from misfolding and aggregation. P4HB operates as part of a larger multi-protein complex that assists in maintaining protein structure under stress conditions in the cell. Its activity ensures protein stability and proper cellular function important for cell viability and health.

Pathways	P4HB plays a central role in the unfolded protein response (UPR) and oxidative protein folding pathway. It interacts with proteins such as calnexin and calreticulin through its involvement in these pathways. During oxidative protein folding P4HB introduces disulfide bonds into nascent proteins while removing incorrect ones ensuring efficient protein quality control.
Associated diseases and disorders	P4HB alteration has connections to diseases like cancer and neurodegenerative disorders. Its overexpression and activity can promote tumor growth by aiding cancerous cell survival through protein homeostasis. Moreover P4HB's association with amyloid precursor protein impacts the progression of Alzheimer's disease. These connections make P4HB a relevant target for research into therapeutic interventions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

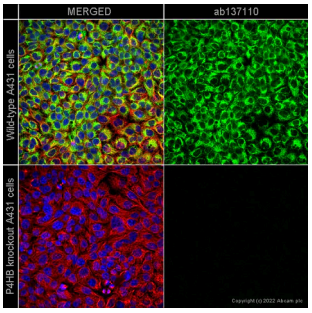
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

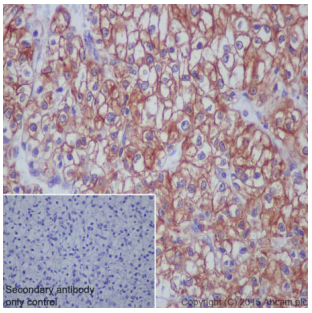
17 product images



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

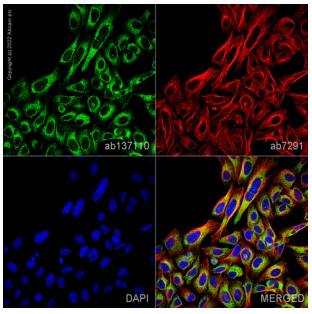
P4HB Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-P4HB antibody

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)

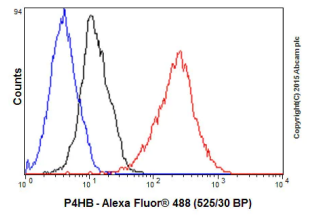
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney carcinoma tissue labelling P4HB with purified ab137110 at a dilution of 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) 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)

P4HB Immunocytochemistry/ Immunofluorescence staining of U2OS cells using rabbit Anti-P4HB antibody

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.

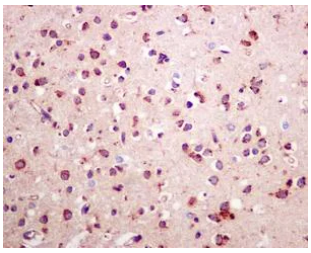


Flow Cytometry (Intracellular) - Anti-P4HB antibody [EPR9499] (ab137110)

P4HB Flow Cytometry (Intracellular) staining using rabbit Anti-P4HB antibody

Intracellular Flow Cytometry analysis ofHeLa 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

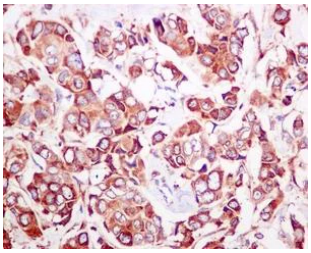
monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



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

P4HB Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-P4HB antibody

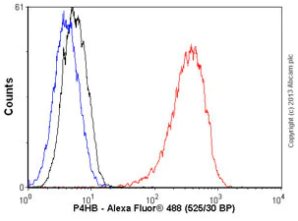
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human brain tissue labelling P4HB with unpurified ab137110 at a dilution of 1/100.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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

P4HB Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-P4HB antibody

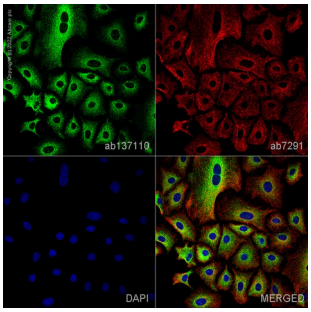
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling P4HB with unpurified ab137110 at a dilution of 1/100.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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

P4HB Flow Cytometry (Intracellular) staining using rabbit Anti-P4HB antibody

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/1x10^6 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.

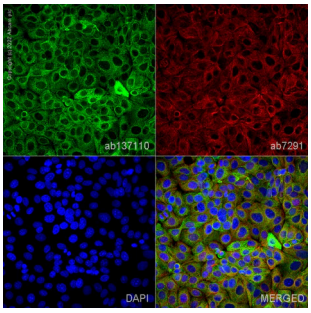


Immunocytochemistry/ Immunofluorescence - Anti-P4HB antibody [EPR9499] (ab137110)

P4HB Immunocytochemistry/ Immunofluorescence staining of A549 cells using rabbit Anti-P4HB antibody

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

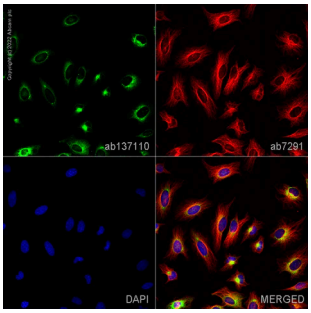
Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 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)

P4HB Immunocytochemistry/ Immunofluorescence staining of MCF7 cells using rabbit Anti-P4HB antibody

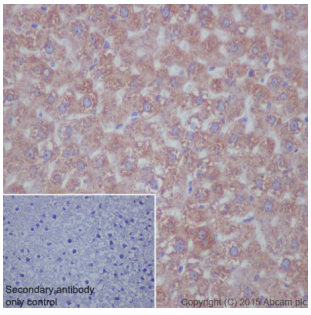
ab137110 staining P4HB in MCF7 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab137110 at 1µg/ml 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.



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

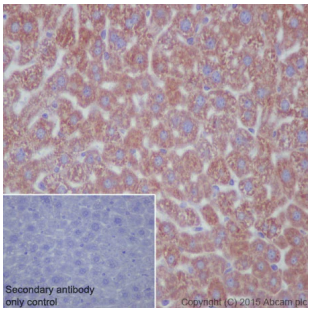
P4HB Immunocytochemistry/ Immunofluorescence staining of HeLa cells using rabbit Anti-P4HB antibody

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.



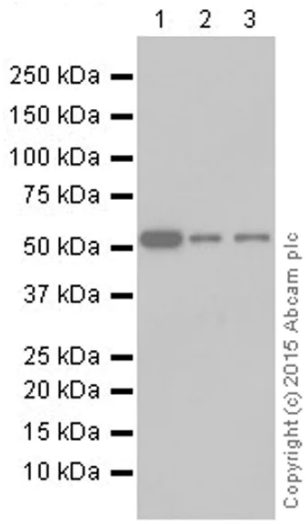
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-P4HB antibody [EPR9499] (ab137110)

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)

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.



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

P4HB Western blot staining using rabbit Anti-P4HB antibody

Blocking and dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-P4HB antibody [EPR9499] (ab137110) at 1/10000 dilution

Lane 1:
HepG2 whole cell lysate at 10 µg

Lane 2:
HeLa whole cell lysate at 10 µg

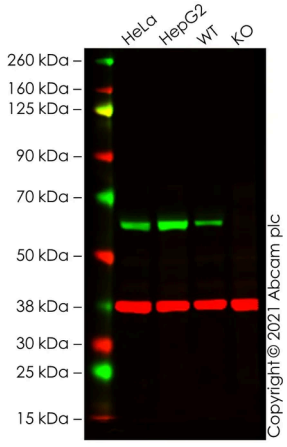
Lane 3:
HEK293 whole cell lysate at 10 µg

Secondary

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

Predicted band size: 57 kDa

Observed band size: 57 kDa



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

P4HB Western blot staining using rabbit Anti-P4HB antibody

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

Lane 1:
HeLa cell lysate at 20 µg

Lane 2:
HepG2 cell lysate at 20 µg

Lane 2:
Western blot - Human P4HB knockout A-431 cell line (ab261887)

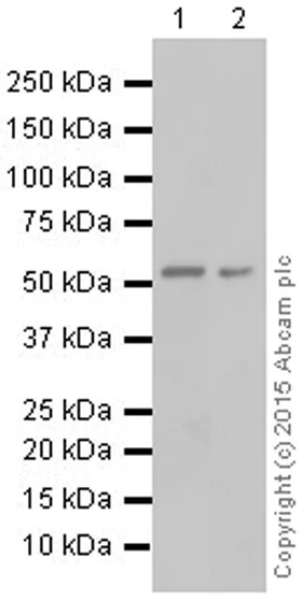
Lane 3:
Wild-type A431 cell lysate at 20 µg

Lane 4:
P4HB knockout A431 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 57 kDa

Observed band size: 60 kDa



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

P4HB Western blot staining using rabbit Anti-P4HB antibody

Blocking and dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-P4HB antibody [EPR9499] (ab137110) at 1/10000 dilution

Lane 1:
Raw264.7 whole cell lysate at 10 µg

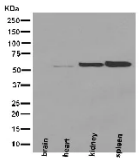
Lane 2:
PC-12 whole cell lysate at 10 µg

Secondary

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

Predicted band size: 57 kDa

Observed band size: 57 kDa



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

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

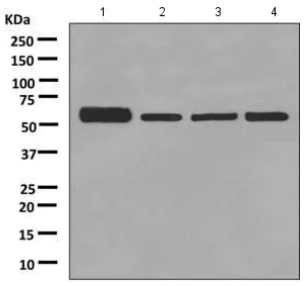
Lane 1:
Mouse brain tissue lysate

Lane 2:
Mouse heart tissue lysate

Lane 3:
Mouse kidney tissue lysate

Lane 4:
Mouse spleen tissue lysate

Predicted band size: 57 kDa



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

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

Lane 1:
HepG2 cell lysate at 10 µg

Lane 2:
HeLa cell lysate at 10 µg

Lane 3:
293T cell lysate at 10 µg

Lane 4:
A431 cell lysate at 10 µg

Secondary

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

Developed using the ECL technique.

Predicted band size: 57 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.