abcam

Product datasheet

Anti-Collagen VI antibody [EPR17072] ab182744

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Collagen VI antibody [EPR17072]
Description	Rabbit monoclonal [EPR17072] to Collagen VI
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IHC-P, mIHC
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human skeletal muscle, Human placenta, Human fetal brain, Human fetal heart, Human fetal kidney, Human fetal spleen, Mouse heart, Mouse kidney, Mouse spleen, Rat kidney and Rat spleen lysates; HEK293T, WI-38 and NIH/3T3 whole cell lysates. IHC-P: Human liver, Human cardiac muscle, Mouse kidney and Rat stomach tissues. mIHC: Human liver and Human prostate gland tissues, human breast. ICC/IF: HeLa cells.
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR17072
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab182744 in the following tested applications.

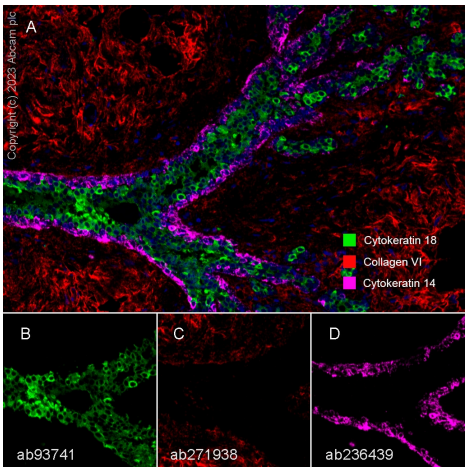
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ (1)	1/2000. Detects a band of approximately 147 kDa (predicted molecular weight: 109 kDa).
ICC/IF		1/200.
IHC-P	★★★★★ (2)	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
mIHC		1/500 - 1/1000.

Target

Function	Collagen VI acts as a cell-binding protein.
Involvement in disease	Defects in COL6A1 are a cause of Bethlem myopathy (BM) [MIM:158810]. BM is a rare autosomal dominant proximal myopathy characterized by early childhood onset (complete penetrance by the age of 5) and joint contractures most frequently affecting the elbows and ankles. Defects in COL6A1 are a cause of Ullrich congenital muscular dystrophy (UCMD) [MIM:254090]; also known as Ullrich scleroatonic muscular dystrophy. UCMD is an autosomal recessive congenital myopathy characterized by muscle weakness and multiple joint contractures, generally noted at birth or early infancy. The clinical course is more severe than in Bethlem myopathy.
Sequence similarities	Belongs to the type VI collagen family. Contains 3 VWFA domains.
Post-translational modifications	Prolines at the third position of the tripeptide repeating unit (G-X-Y) are hydroxylated in some or all of the chains.
Cellular localization	Secreted > extracellular space > extracellular matrix.

Images



Multiplex immunohistochemistry - Anti-Collagen VI antibody [EPR17072] (ab182744)

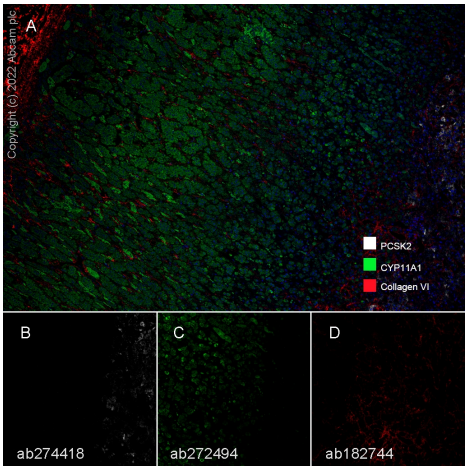
This data was developed using the same antibody clone in a different buffer formulation (**ab271938**).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast labelling Cytokeratin 18 with **ab93741** at 1/200 dilution (1.02 µg/mL) (B), Collagen VI with **ab271938** at 1/500 dilution (2.084 µg/ml) (C) and Cytokeratin 14 with **ab236439** at 1/2000 dilution (0.519 µg/ml) (D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

Panel A: merged staining of anti-Cytokeratin 14 (magenta; Opal™690), anti-Cytokeratin 18 (green; Opal™520) and anti-Collagen VI (red; Opal™570) on human breast.
Panel B: anti-Cytokeratin 18 stained on luminal epithelial cells.
Panel C: anti-Collagen VI stained on stroma.
Panel D: anti-Cytokeratin 14 stained on myoepithelial cells.

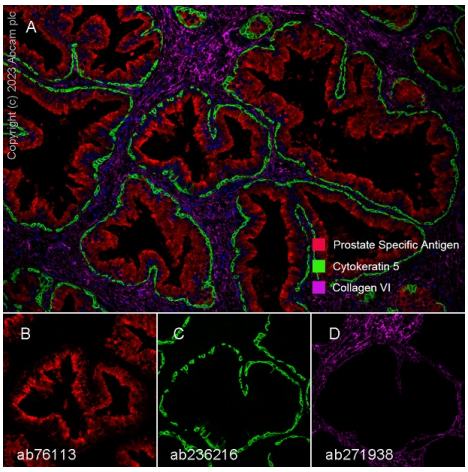
The section was incubated in three rounds of staining: in the order of **ab236439** for 30 mins, **ab93741** for 10 mins, and **ab271938** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



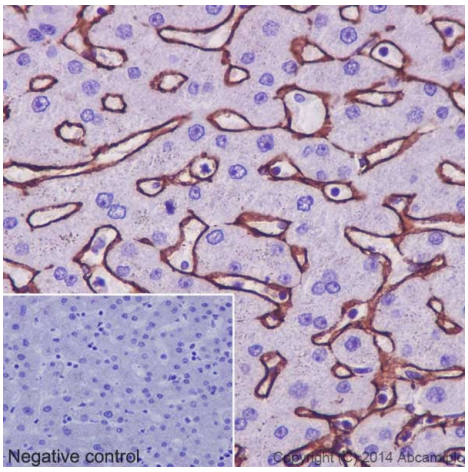
Multiplex immunohistochemistry - Anti-Collagen VI antibody [EPR17072] (ab182744)

Fluorescence multiplex immunohistochemical analysis of human adrenal gland (formalin-fixed paraffin-embedded section). Panel A shows merged staining of anti-PCSK2 stained on adrenal medulla (**ab274418**; gray; Opal™690) at 1:2000 (0.263 µg/ml) [Panel B], anti-CYP11A1 stained on adrenal cortex (**ab272494**; green; Opal™520) at 1:10000 (0.053 µg/ml) [Panel C], and anti-Collagen VI stained on extracellular matrix (ab182744; red; Opal™570) at 1:1000 (1.518 µg/ml) [Panel D] on human adrenal gland. DAPI was used as a nuclear counter stain. Followed by Opal Polymer HRP Ms + Rb secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. The section was incubated in three rounds of staining: in the order of **ab274418**, **ab272494**, and ab182744 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used.



Multiplex immunohistochemistry - Anti-Collagen VI antibody [EPR17072] (ab182744)

Fluorescence multiplex immunohistochemical analysis of human prostate gland tissue (formalin/PFA-fixed paraffin-embedded section). Panel A: merged staining of anti-Collagen VI (**ab271938**, magenta; Opal™690), anti-Cytokeratin 5 (**ab236216**, green; Opal™520) and anti-Prostate Specific Antigen (**ab76113**, red; Opal™570) on human prostate gland tissue. Panel B: anti-Prostate Specific Antigen stained on luminal cells. Panel C: anti-Cytokeratin 5 stained on basal cells. Panel D: anti-Collagen VI stained on stroma. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of **ab271938** (1/500), **ab236216** (1/400), and **ab76113** (1/2000) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) was used for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

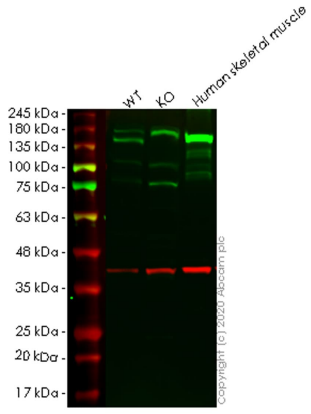


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen VI antibody [EPR17072] (ab182744)

Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling Collagen VI with ab182744 at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Positive staining around sinusoidal endothelial basement membranes is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Collagen VI antibody [EPR17072] (ab182744)

All lanes : Anti-Collagen VI antibody [EPR17072] (ab182744) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : COL6A1 knockout HEK293T cell lysate
Lane 3 : Human skeletal muscle tissue lysate

Lysates/proteins at 20 µg per lane.

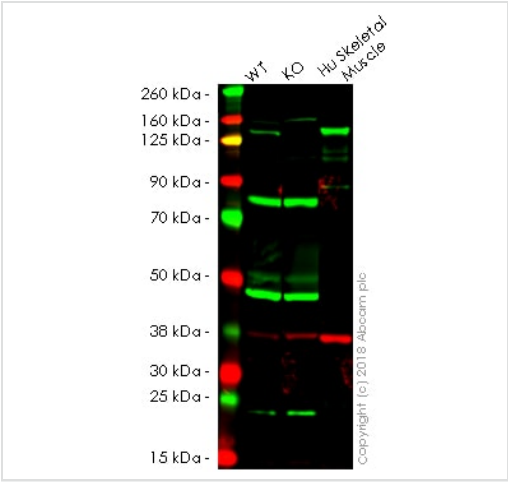
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 109 kDa
Observed band size: 136 kDa

Lanes 1-3: Merged signal (red and green). Green - ab182744 observed at 136 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab182744 Anti-Collagen VI antibody [EPR17072] was shown to specifically react with Collagen VI antibody in wild-type HEK293T

cells. Loss of signal was observed when knockout cell line **ab265060** (knockout cell lysate **ab256879**) was used. Wild-type and Collagen VI antibody knockout samples were subjected to SDS-PAGE. ab182744 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Collagen VI antibody [EPR17072] (ab182744)

All lanes : Anti-Collagen VI antibody [EPR17072] (ab182744)

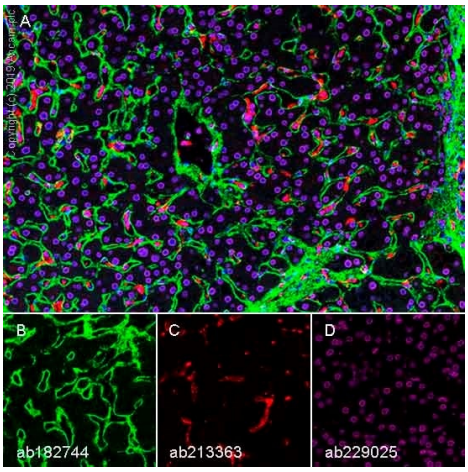
Lane 1 : Wild-type HEK293 whole cell lysate
Lane 2 : COL6A1 knockout HEK293 whole cell lysate
Lane 3 : Human Skeletal Muscle whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 109 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab182744 observed at 109 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab182744 was shown to recognize Collagen VI in wild-type HEK293 cells as signal was lost at the expected MW in COL6A1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and COL6A1 knockout samples were subjected to SDS-PAGE. Ab182744 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Multiplex immunohistochemistry - Anti-Collagen VI antibody [EPR17072] (ab182744)

Multiplex immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue.

Panel A: Merged staining of Collagen VI (ab182744; green), anti-CD68 (**ab213363**; red) and anti-Lamin B1 (**ab229025**; magenta).

Panel B: Anti-Collagen VI (green) stained on extracellular matrix.

Panel C: Anti-CD68 (red) stained on Kupffer cells.

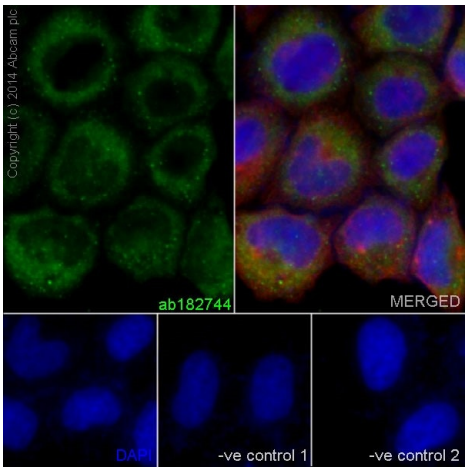
Panel D: Anti-Lamin B1 (magenta) stained on nuclear envelope.

Key protocol steps: The section was incubated in three rounds of staining with ab182744 (1/1000 dilution), **ab213363** (1/1000 dilution) and **ab229025** (1/4000 dilution) for 30 mins at room temperature. Each round was followed by tyramide signal amplification with the appropriate fluorophore.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

DAPI was used as a nuclear counter stain. A ready-to-use anti-Rabbit and Mouse Polymer HRP was used as a secondary.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

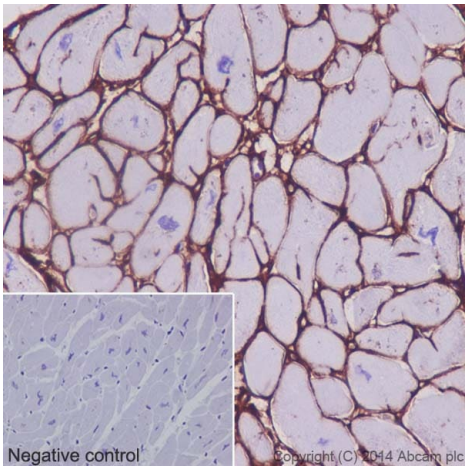


Immunocytochemistry/ Immunofluorescence - Anti-Collagen VI antibody [EPR17072] (ab182744)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Collagen VI with ab182744 at 1/200 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Cytoplasm staining on HeLa cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:-

-ve control1 - ab182744 at 1/200 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2 - **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.

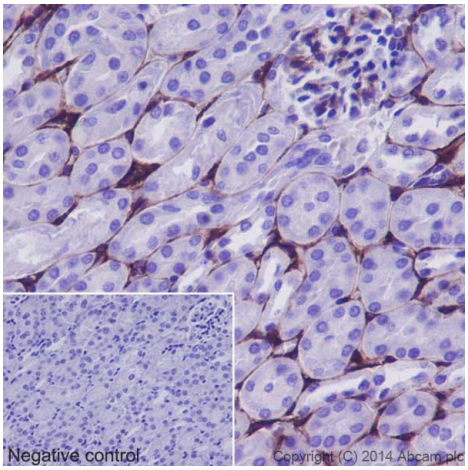


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen VI antibody [EPR17072] (ab182744)

Immunohistochemical analysis of paraffin-embedded Human cardiac muscle tissue labeling Collagen VI with ab182744 at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Positive staining on Human cardiac sarcolemma and interstitium is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

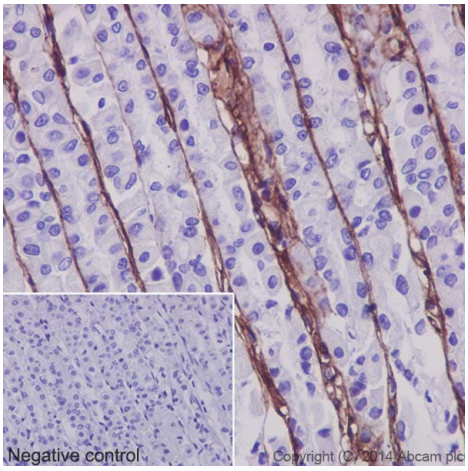


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen VI antibody [EPR17072] (ab182744)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling Collagen VI with ab182744 at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Positive staining around basement membranes of Mouse renal tubules is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

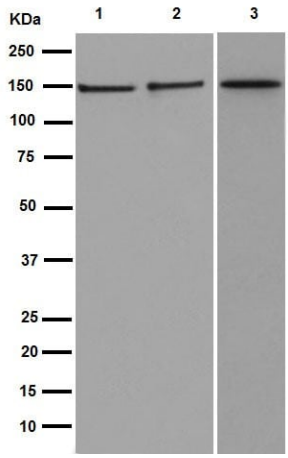


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen VI antibody [EPR17072] (ab182744)

Immunohistochemical analysis of paraffin-embedded Rat stomach tissue labeling Collagen VI with ab182744 at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Positive staining around Rat gastric epithelial basement membranes is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Collagen VI antibody [EPR17072] (ab182744)

All lanes : Anti-Collagen VI antibody [EPR17072] (ab182744) at 1/20000 dilution

Lane 1 : Human skeletal muscle lysate
Lane 2 : WI-38 (Human fetal lung fibroblast cells) whole cell lysate
Lane 3 : Human placenta lysate

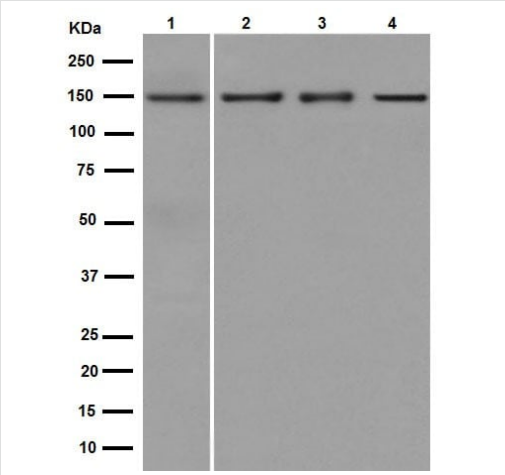
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 109 kDa
Observed band size: 147 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Due to posttranslational modifications, observed MW is greater than the predicted MW.



Western blot - Anti-Collagen VI antibody [EPR17072] (ab182744)

All lanes : Anti-Collagen VI antibody [EPR17072] (ab182744) at 1/2000 dilution

Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal heart lysate
Lane 3 : Human fetal kidney lysate
Lane 4 : Human fetal spleen lysate

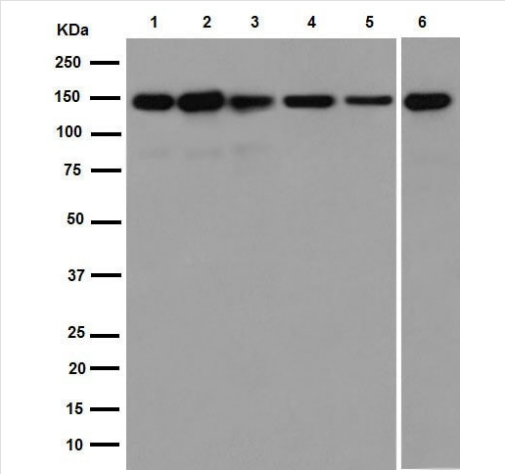
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 109 kDa
Observed band size: 147 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Due to posttranslational modifications, observed MW is greater than the predicted MW.



Western blot - Anti-Collagen VI antibody [EPR17072] (ab182744)

All lanes : Anti-Collagen VI antibody [EPR17072] (ab182744) at 1/2000 dilution

Lane 1 : Mouse heart lysate
Lane 2 : Mouse kidney lysate
Lane 3 : Mouse spleen lysate
Lane 4 : Rat kidney lysate
Lane 5 : Rat spleen lysate
Lane 6 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 109 kDa

Observed band size: 147 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Due to posttranslational modifications, observed MW is greater than the predicted MW.

Anti-Collagen VI antibody [EPR17072] (ab182744)

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