abcam

Product datasheet

Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free ab229450

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR17072] to Collagen VI - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, 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	ab229450 is the carrier-free version of **ab182744**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17072
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab229450 in the following tested applications.

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

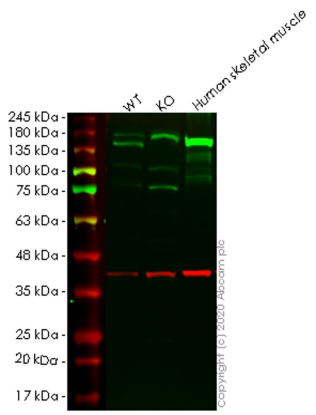
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 147 kDa (predicted molecular weight: 109 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
mIHC		Use at an assay dependent concentration.

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



Western blot - Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free (ab229450)

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

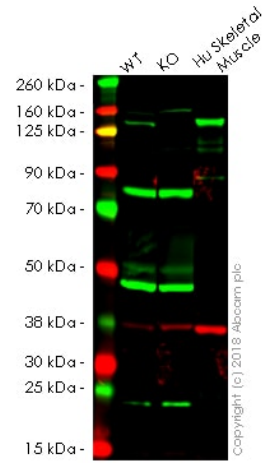
Predicted band size: 109 kDa
Observed band size: 136 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab182744**).

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] - Low endotoxin, Azide free (ab229450)

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

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

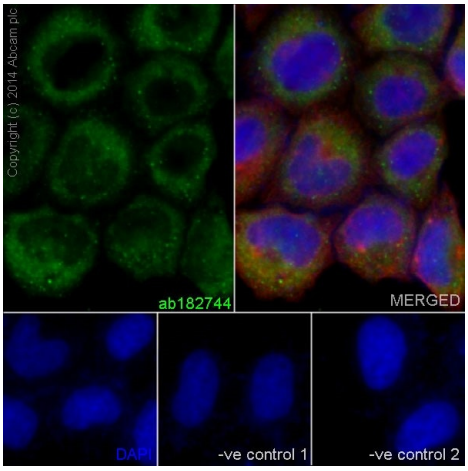
Lysates/proteins at 20 µg per lane.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182744**).



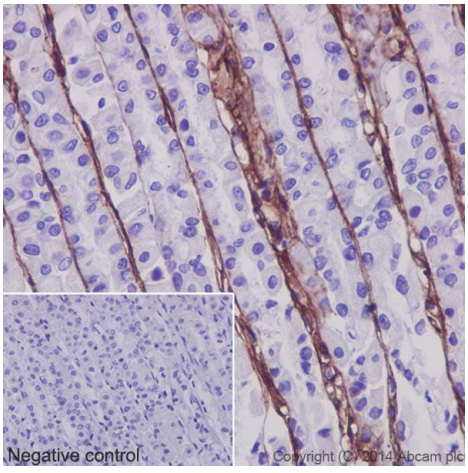
Immunocytochemistry/ Immunofluorescence - Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free (ab229450)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182744**).



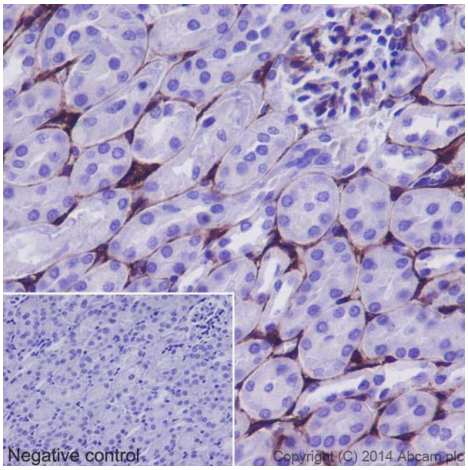
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free (ab229450)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182744**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



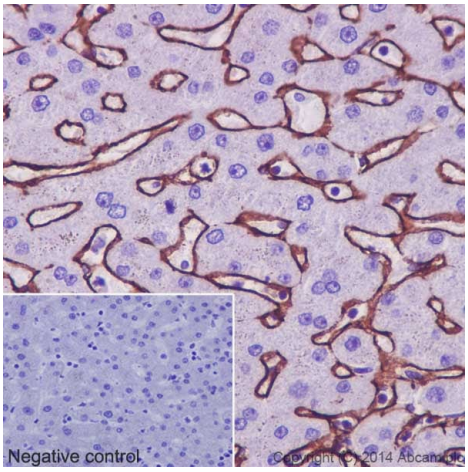
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free (ab229450)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182744**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



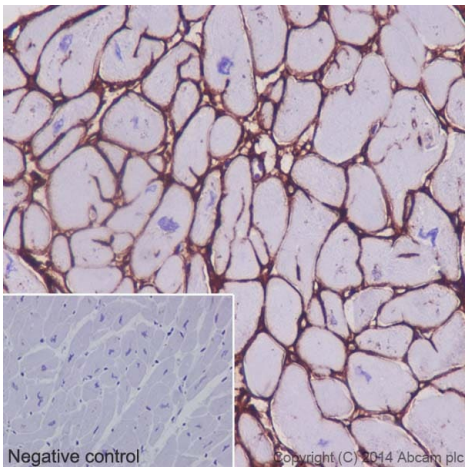
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free (ab229450)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab182744**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



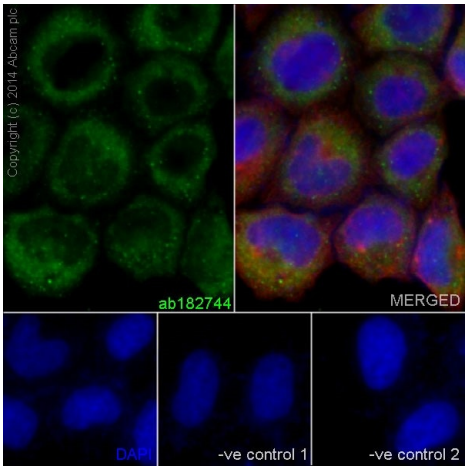
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free (ab229450)

This IHC data was generated using the same anti-Collagen VI antibody clone, EPR17072, in a different buffer formulation (cat# **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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



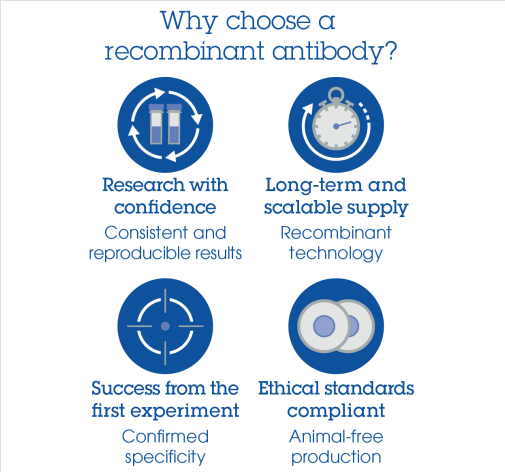
Immunocytochemistry/ Immunofluorescence - Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free (ab229450)

This ICC/IF data was generated using the same anti-Collagen VI antibody clone, EPR17072, in a different buffer formulation (cat# **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.



Anti-Collagen VI antibody [EPR17072] - Low endotoxin, Azide free (ab229450)

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