abcam

Product datasheet

Anti-Collagen XVII antibody [EPR18614] ab184996



★★★★★ 4 Abreviews 25 References 12 Images

Overview

Product name Anti-Collagen XVII antibody [EPR18614]

Description Rabbit monoclonal [EPR18614] to Collagen XVII

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, IP Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: A431 whole cell lysate, human, mouse and rat skin lysates. IHC-P: Human esophagus,

mouse skin and rat skin tissues. ICC/IF: HaCaT and A431 cells. IP: A431 whole cell lysate.

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, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR18614

Isotype ΙgG

Applications

The Abpromise quarantee

Our **Abpromise guarantee** covers the use of ab184996 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	**** <u>(1)</u>	1/1000. Detects a band of approximately 180, 130 kDa (predicted molecular weight: 150 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	*** <u>*</u>	1/100.
IP	★★★★★ (1)	1/50.

Target

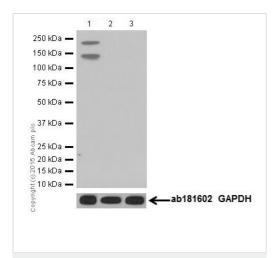
Relevance

Unlike most collagens, collagen XVII is a transmembrane protein. Collagen XVII is a structural component of hemidesmosomes, multiprotein complexes at the dermal epidermal basement membrane zone that mediate adhesion of keratinocytes to the underlying membrane. Mutations in the gene coding for collagen XVII are associated with both generalized atrophic benign and junctional epidermolysis bullosa. Two homotrimeric forms of type XVII collagen exist. The full length form is the transmembrane protein. A soluble form, referred to as either ectodomain or LAD 1, is generated by proteolytic processing of the full length form. Two transcript variants, one resulting from alternative splicing in the 3' UTR, have been identified for this gene.

Cellular localization

Cell junction, hemidesmosome. Membrane; Single-pass type II membrane protein. Note=Localized along the plasma membrane of the hemidesmosome. 120 kDa linear IgA disease antigen and 97 kDa linear IgA disease antigen: Secreted, extracellular space, extracellular matrix, basement membrane.

Images



Western blot - Anti-Collagen XVII antibody [EPR18614] (ab184996) **All lanes**: Anti-Collagen XVII antibody [EPR18614] (ab184996) at 1/5000 dilution

Lane 1: Human skin lysate

Lane 2: Human fetal liver lysate

Lane 3: Human fetal lung lysate

Lysates/proteins at 10 µg per lane.

Secondary

 $\textbf{All lanes:} \ \, \textbf{Anti-Rabbit lgG (HRP)}, \ \, \textbf{specific to the non-reduced form}$

of IgG at 1/10000 dilution

Predicted band size: 150 kDa

Observed band size: 130,180 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 15161638).

250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

Western blot - Anti-Collagen XVII antibody [EPR18614] (ab184996) Anti-Collagen XVII antibody [EPR18614] (ab184996) at 1/5000 dilution + A431 (Human epidermoid carcinoma) whole cell lysate at $10~\mu g$

Secondary

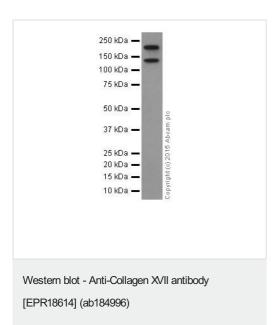
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 150 kDa **Observed band size:** 130,180 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 15161638).



Anti-Collagen XVII antibody [EPR18614] (ab184996) at 1/2000 dilution + Mouse skin lysate at 10 μg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 150 kDa **Observed band size:** 130,180 kDa

Exposure time: 2 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 15161638).

250 kDa —

150 kDa —

100 kDa —

75 kDa —

50 kDa —

37 kDa —

25 kDa —

20 kDa —

15 kDa —

10 kDa —

10 kDa —

Western blot - Anti-Collagen XVII antibody [EPR18614] (ab184996) Anti-Collagen XVII antibody [EPR18614] (ab184996) at 1/1000 dilution + Rat skin lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

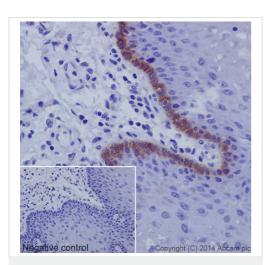
Predicted band size: 150 kDa

Observed band size: 130,180 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 15161638).



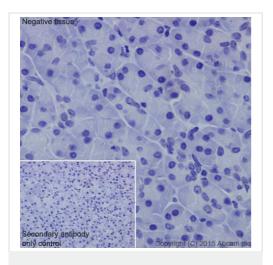
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Collagen XVII antibody
[EPR18614] (ab184996)

Immunohistochemical analysis of paraffin-embedded Human esophagus tissue labeling Collagen XVII with ab184996 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on squamous epithelial cells of Human esophagus is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

J Biol Chem. 1991. 266, 24064-24069.

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

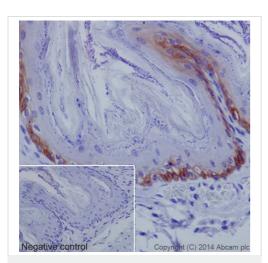


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Collagen XVII antibody [EPR18614] (ab184996)

Immunohistochemical analysis of paraffin-embedded Human pancreas tissue labeling Collagen XVII with ab184996 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Negative on Human pancreas. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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

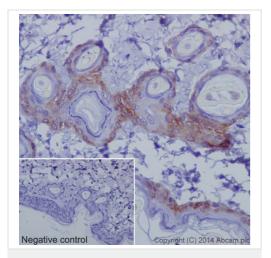


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Collagen XVII antibody [EPR18614] (ab184996)

Immunohistochemical analysis of paraffin-embedded mouse skin tissue labeling Collagen XVII with ab184996 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic and membrane staining on squamous epithelial cells of mouse skin is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Collagen XVII antibody [EPR18614] (ab184996)

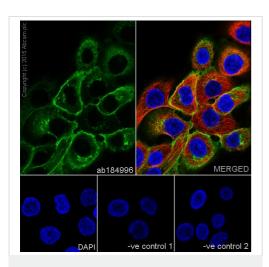
Immunohistochemical analysis of paraffin-embedded rat skin tissue labeling Collagen XVII with ab184996 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on epithelial cells of rat skin is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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

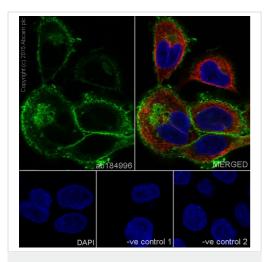


Immunocytochemistry/ Immunofluorescence - Anti-Collagen XVII antibody [EPR18614] (ab184996)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HaCaT (Human keratinocyte cells) cells labeling Collagen XVII with ab184996 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic and membranous staining on HaCaT cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab184996 at 1/100 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2:. <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

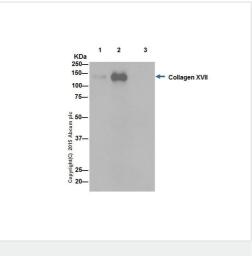


Immunocytochemistry/ Immunofluorescence - Anti-Collagen XVII antibody [EPR18614] (ab184996)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A431 (Human epidermoid carcinoma) cells labeling Collagen XVII with ab184996 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic and membranous staining on A431 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab184996 at 1/100 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



Immunoprecipitation - Anti-Collagen XVII antibody [EPR18614] (ab184996)

Collagen XVII was immunoprecipitated from 1mg of A431 (Human epidermoid carcinoma) whole cell lysate with ab184996 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab184996 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

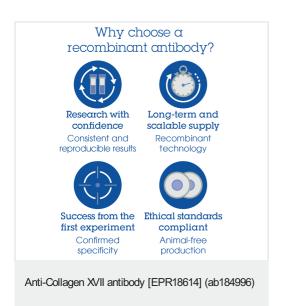
Lane 1: A431 whole cell lysate 10ug (Input).

Lane 2: ab184996 IP in A431 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab184996 in A431 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.



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