abcam

Product datasheet

Anti-Collagen III antibody [EPR17673] ab184993

Recombinant RabMAb

47 References 16 Images

Overview

Product name Anti-Collagen III antibody [EPR17673]

Description Rabbit monoclonal [EPR17673] to Collagen III

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF, IP, WB

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human fetal liver, fetal kidney, skin, skeletal muscle and heart lysates; HeLa, MCF7, HaCaT

> and A431 whole cell lysates. Mouse skeletal muscle tissue lysates, rat skeletal muscle tissue lysates, mouse heart tissue lysate, C6, RAW264.7, PC-12 and NIH/3T3 whole cell lysates. C6, RAW264.7 and PC-12 whole cell fresh lysate. ICC/IF: HeLa, MCF7, PC-12 and RAW 264.7 cells

Flow Cyt (intra): HeLa, PC-12 and RAW 264.7 cells IP: HeLa 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: 0.05% BSA, 40% Glycerol, PBS

Purity Protein A purified

Clonality Monoclonal

Clone number EPR17673

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab184993 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)		1/50.
ICC/IF		1/100.
IP		1/30.
WB		1/1000. Detects a band of approximately 150 kDa (predicted molecular weight: 139 kDa).

Target

Function

Involvement in disease

Collagen type III occurs in most soft connective tissues along with type I collagen.

Defects in COL3A1 are a cause of Ehlers-Danlos syndrome type 3 (EDS3) [MIM:130020]; also known as benign hypermobility syndrome. EDS is a connective tissue disorder characterized by hyperextensible skin, atrophic cutaneous scars due to tissue fragility and joint hyperlaxity. EDS3 is a form of Ehlers-Danlos syndrome characterized by marked joint hyperextensibility without skeletal deformity.

Defects in COL3A1 are the cause of Ehlers-Danlos syndrome type 4 (EDS4) [MIM:130050]. EDS is a connective tissue disorder characterized by hyperextensible skin, atrophic cutaneous scars due to tissue fragility and joint hyperlaxity. EDS4 is the most severe form of the disease. It is characterized by the joint and dermal manifestations as in other forms of the syndrome, characteristic facial features (acrogeria) in most patients, and by proneness to spontaneous rupture of bowel and large arteries. The vascular complications may affect all anatomical areas. Defects in COL3A1 are a cause of susceptibility to aortic aneurysm abdominal (AAA) [MIM:100070]. AAA is a common multifactorial disorder characterized by permanent dilation of the abdominal aorta, usually due to degenerative changes in the aortic wall. Histologically, AAA is characterized by signs of chronic inflammation, destructive remodeling of the extracellular matrix,

Sequence similarities

Belongs to the fibrillar collagen family.

Contains 1 fibrillar collagen NC1 domain.

and depletion of vascular smooth muscle cells.

Contains 1 VWFC domain.

Post-translational modifications

Proline residues at the third position of the tripeptide repeating unit (G-X-Y) are hydroxylated in

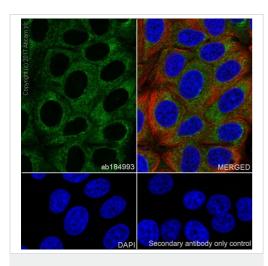
some or all of the chains.

O-linked glycan consists of a Glc-Gal disaccharide bound to the oxygen atom of a post-

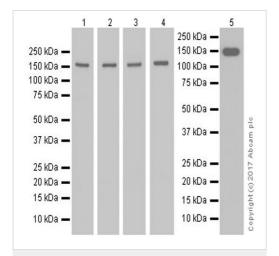
translationally added hydroxyl group.

Cellular localization

Secreted > extracellular space > extracellular matrix.



Immunocytochemistry/ Immunofluorescence - Anti-Collagen III antibody [EPR17673] (ab184993)



Western blot - Anti-Collagen III antibody [EPR17673] (ab184993)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF7 (human breast adenocarcinoma cell line) cells labeling Collagen III with ab184993 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on MCF7 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

All lanes : Anti-Collagen III antibody [EPR17673] (ab184993) at 1/1000 dilution

Lane 1 : Human fetal liver lysate at 20 μg

Lane 2: Human fetal kidney lysate at 20 µg

Lane 3: HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 4 : MCF7 (human breast adenocarcinoma cell line) whole cell lysate at 20 µg

Lane 5: HaCaT (human keratinocyte cell line) whole cell lysate at 10 μg

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 139 kDa **Observed band size:** 150 kDa

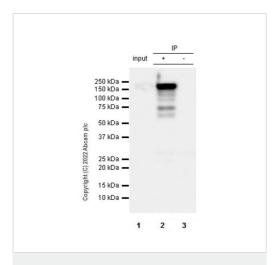
Exposure time : Lane 1: 3 minutes; Lane 2: 15 seconds; Lane 3: 3

seconds; Lanes 4-5: 30 seconds.

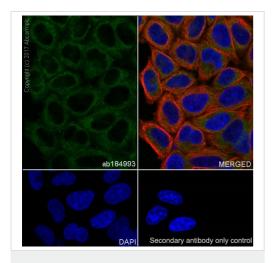
Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been

described in the literature (PMID: 26017148).



Immunoprecipitation - Anti-Collagen III antibody [EPR17673] (ab184993)



Immunocytochemistry/ Immunofluorescence - Anti-Collagen III antibody [EPR17673] (ab184993)

Collagen III was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab184993 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab184993 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (Input).

Lane 2: ab184993 IP in HeLa whole cell lysate.

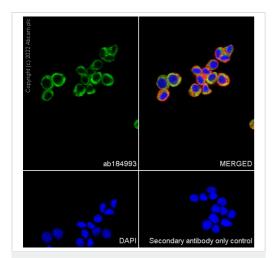
Lane 3: Rabbit monoclonal $\lg G$ (<u>ab172730</u>) instead of ab184993 in HeLa whole cell lysate .

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 5.5 seconds.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Collagen III with ab184993 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HeLa cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Collagen III antibody [EPR17673] (ab184993)

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DAPI

Secondary antibody only control

Immunocytochemistry/ Immunofluorescence - Anti-Collagen III antibody [EPR17673] (ab184993)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized PC-12 (rat adrenal gland pheochromocytoma small irregularly shaped cells) cell line labeling Collagen III with ab184993 at 1/50 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed secondary antibody (ab150081) at 1/1000 dilution (green).Confocal image showing cytoplasmic staining in PC-12 cell line.

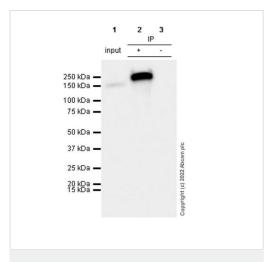
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary is Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody (ab150081) at 1/1000 dilution.

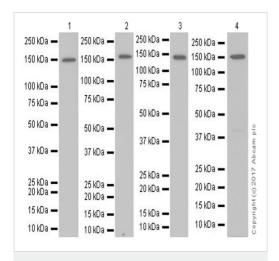
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) cell line labeling Collagen III with ab184993 at 1/50 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody (ab150081) at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in RAW 264.7 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary is Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody (ab150081) at 1/1000 dilution.



Immunoprecipitation - Anti-Collagen III antibody [EPR17673] (ab184993)



Western blot - Anti-Collagen III antibody [EPR17673] (ab184993)

Collagen III was immunoprecipitated from 0.35 mg of RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate with ab184993 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab184993 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: RAW 264.7 whole cell lysate 10 µg (Input).

Lane 2: ab184993 IP in RAW 264.7 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab184993 in RAW 264.7 whole cell lysate.

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

Exposure time: 3.25 seconds.

Lysate was freshly made and used immediately to minimize protein degradation.

All lanes : Anti-Collagen III antibody [EPR17673] (ab184993) at 1/1000 dilution

Lane 1: Human skin lysate

Lane 2: Human skeletal muscle lysate

Lane 3: Human heart lysate

Lane 4: A431 (human epidermoid carcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Lanes 2-4: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Developed using the ECL technique.

Predicted band size: 139 kDa **Observed band size:** 150 kDa

Exposure time: Lanes 1-3: 30 seconds; Lane 4: 5 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

1 2 3 4 7 250 kDa-250 kDa-250 kDa= 250 kDa= 150 kDa -150 kDa-150 kDa= 150 kDa= 100 kDa= 100 kDa-100 kDa-100 kDa= 75 kDa= 75 kDa-75 kDa-75 kDa= 50 kDa= 50 kDa-50 kDa-50 kDa -37 kDa-37 kDa-37 kDa= 37 kDa-25 kDa = 20 kDa = 25 kDa-25 kDa = 20 kDa = 25 kDa-20 kDa-20 kDa-15 kDa= 15 kDa-15 kDa-15 kDa-10 kDa-10 kDa-10 kDa-10 kDa-Frozen lysates Fresh lysates

Western blot - Anti-Collagen III antibody [EPR17673] (ab184993)

The molecular weight observed is consistent with what has been described in the literature (PMID: 26017148).

All lanes: Anti-Collagen III antibody [EPR17673] (ab184993) at 1/1000 dilution

Lane 1: C6 (rat glial tumor glial cell), whole cell lysate

Lane 2: RAW264.7 (mouse Abelson murine leukemia virus-

induced tumor macrophage), whole cell lysate

Lane 3: PC-12 (rat adrenal gland pheochromocytoma), whole cell

lysate

Lane 4: NIH/3T3 (mouse embryonic fibroblast), whole cell lysate

Lane 5: C6 (rat glial tumor glial cell), whole cell fresh lysate

Lane 6: RAW264.7 (mouse Abelson murine leukemia virus-

induced tumor macrophage), whole cell fresh lysate

Lane 7: PC-12 (rat adrenal gland pheochromocytoma), whole cell

fresh lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 139 kDa Observed band size: 150 kDa

Exposure time : Lanes 1-4: 92 seconds; Lanes 5-6: 48 seconds;

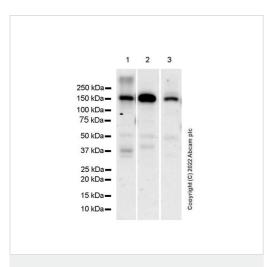
Lane 7: 37 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

In lane 1-4, the lysates were stored at -80? prior to Western Blotting.

In lane 5-7, the lysates were freshly made and used for Western

Blotting immediately to minimize protein degradation.



Western blot - Anti-Collagen III antibody [EPR17673] (ab184993)

All lanes : Anti-Collagen III antibody [EPR17673] (ab184993) at 1/1000 dilution

Lane 1: Mouse skeletal muscle tissue lysate

Lane 2: Mouse heart tissue lysate

Lane 3: Rat skeletal muscle tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 139 kDa **Observed band size:** 150 kDa

Exposure time: 3 minutes

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

1 2 3
input +
250 kDa 150 kDa 150 kDa 75 kDa
37 kDa 25 kDa 25 kDa 25 kDa 26 kDa 27 kDa 27 kDa 28 kDa 29 kDa 29 kDa 20 kDa 20 kDa 20 kDa -

Immunoprecipitation - Anti-Collagen III antibody [EPR17673] (ab184993)

Collagen III was immunoprecipitated from 0.35 mg of PC-12 (rat adrenal gland pheochromocytoma cell), whole cell lysate with ab184993 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab184993 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: PC-12 whole cell lysate 10 µg (Input).

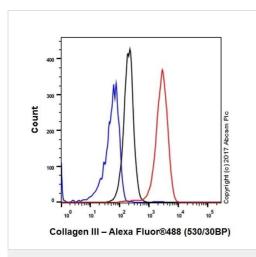
Lane 2: ab184993 IP in PC-12 whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab184993 in PC-12 whole cell lysate.

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

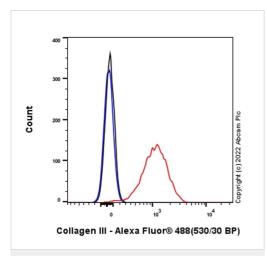
Exposure time: 3.25 seconds.

Lysate was freshly made and used immediately to minimize protein degradation.



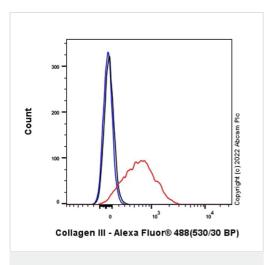
Flow Cytometry (Intracellular) - Anti-Collagen III antibody [EPR17673] (ab184993)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling Collagen Illwith ab184993 at 1/50 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.



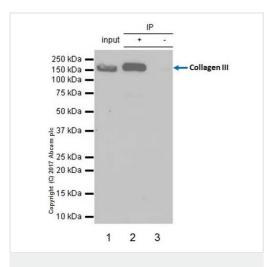
Flow Cytometry (Intracellular) - Anti-Collagen III antibody [EPR17673] (ab184993)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) cell line labeling Collagen III with ab184993 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150081) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Collagen III antibody [EPR17673] (ab184993)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized PC-12 (Rat adrenal gland pheochromocytoma) cell line labeling Collagen III with ab184993 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Collagen III antibody [EPR17673] (ab184993)

Collagen III was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab184993 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab184993 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

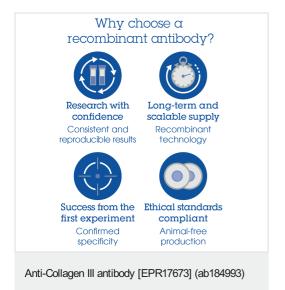
Lane 1: HeLa whole cell lysate 10 µg (Input).

Lane 2: ab184993 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ (<u>ab172730</u>) instead of ab184993 in HeLa whole cell lysate .

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

Exposure time: 10 seconds.



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