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Datasheet for 600-401-105-0.5

Collagen Type III Antibody

Overview

Description:	Anti-Collagen Type III (RABBIT) Antibody - 600-401-105-0.5
Item No.:	600-401-105-0.5
Size:	500 µg
Applications:	Dot Blot, IHC, WB, ELISA, FC, IF, Multiplex
Reactivity:	Human, Bovine, Pig
Host Species:	Rabbit

Product Details

Background:	Rockland produces highly active antibodies and conjugates to collagens. Collagens are highly conserved throughout evolution and are characterized by an uninterrupted ''Glycine-X-Y'' triplet repeat that is a necessary part of the triple helical structure. For these reasons, it is often extremely difficult to generate antibodies with specificities to collagens. The development of 'type' specific antibodies is dependent on NON-DENATURED three-dimensional epitopes. Rockland extensively purifies collagens for immunization from human and bovine placenta and cartilage by limited pepsin digestion and selective salt precipitation. This preparation results in a native conformation of the protein. Antibodies are isolated from rabbit antiserum and are extensively cross-adsorbed by immunoaffinity purification to produce 'type' specific antibodies. Greatly diminished reactivity and selectivity of these antibodies will result if denaturing and reducing conditions are used for SDS-PAGE and immunoblotting.
Synonyms:	rabbit anti-Collagen Type III antibody, Collagen type III alpha 1 antibody, Collagen type III alpha antibody, EDS4A antibody, Ehlers Danlos syndrome type IV, autosomal dominant antibody, Fetal collagen antibody, COL3A1, Collagen alpha-1 (III) chain
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	COL3A1
Reactivity:	Human, Bovine, Pig

Immunogen Type:	Native Protein
Immunogen:	Collagen Type III from human and bovine placenta
Purity/Specificity:	Collagen III Antibody has been prepared by immunoaffinity chromatography using immobilized antigens. Some class-specific anti-collagens may be specific for three-dimensional epitopes which may result in diminished reactivity with denatured collagen or formalin-fixed, paraffin embedded tissues. This antibody reacts with most mammalian Type III collagens and has expected cross-reactivity with Type I and negligible cross reactivity with Type II, IV, V or VI collagens. Non-specific cross-reaction of anti-collagen antibodies with other human serum proteins or non-collagen extracellular matrix proteins has not been tested.
Relevant Links:	• Anti-Collagen IHC Protocol • UniProtKB - P02461 • NCBI - NP_000081.1 • GeneID - 1281

Application Details

Tested Applications:	Dot Blot, IHC, WB
Suggested Applications:	ELISA, FC, IF, Multiplex (Based on references)
Application Note:	Anti-Collagen Type III has been tested by dot Blot, western blot, and IHC and is useful for indirect trapping ELISA for quantitation of antigen in serum using a standard curve, immunoprecipitation, native (non-denaturing, non-dissociating) PAGE, immunohistochemistry, and western blotting for highly sensitive qualitative analysis.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:50,000
FC:	User Optimized
IF:	User Optimized
IHC:	1:50 - 1:200
IP:	1:100
WB:	1:1,000 - 1:10,000

Formulation

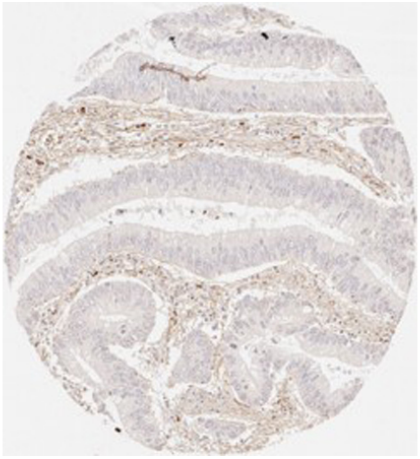
Physical State:	Liquid (sterile filtered)
Concentration:	1.16 mg/mL by UV absorbance at 280 nm

Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

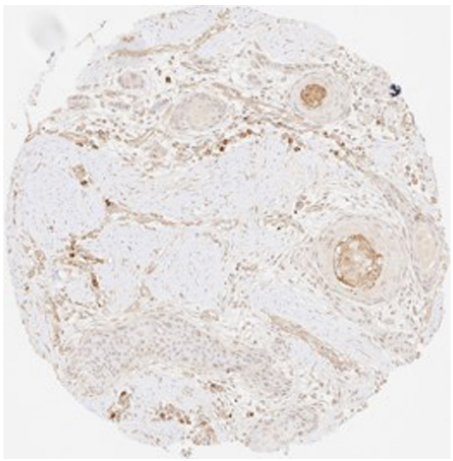
Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	Store vial at 4° C prior to opening. This product is stable at 4° C as an undiluted liquid. Dilute only prior to immediate use. For extended storage, mix with an equal volume of glycerol, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

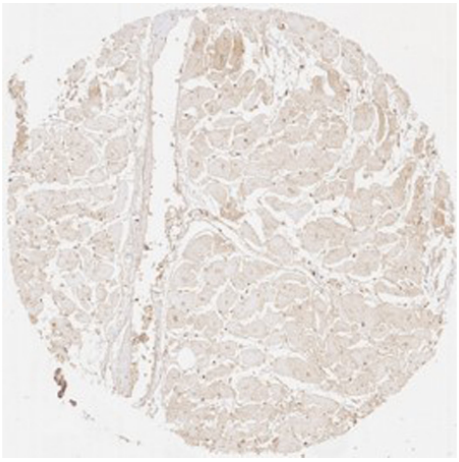
Images



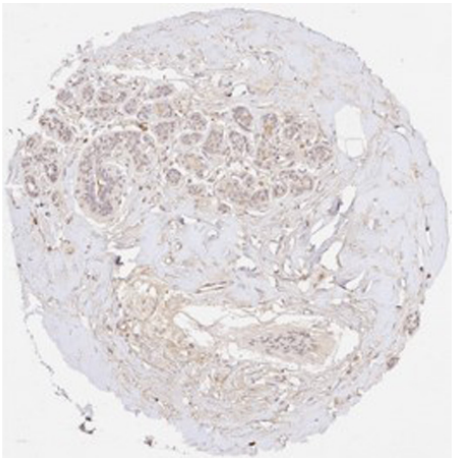
Immunohistochemistry
Immunohistochemistry results of Rabbit Anti-Collagen Type I Antibody. Tissue: human stroma in colorectal cancer. Fixation: FFPE. Antigen Retrieval: HIER using Tris-EDTA-citrate buffer pH 7.8 for 5 min. Blocking: Peroxidase-Blocking Solution for 10 min. Primary Antibody: Anti-Collagen Type I (p/n 600-401-103-0.1) at 1:15 for 1 hr at 37 °C. Secondary Antibody: Dako REAL EnVision Detection Kit, Polymer-HRP, Rabbit/Mouse. Counterstain: Hematoxylin for 15 sec. Substrate: DAB-Chromogen, Rabbit/Mouse. Staining/Results: Fibrillar collagen III staining of the stroma in a colorectal cancer. Independently Validated by antibodies-online GmbH (p/n ABIN7565873/ ABIN5596830/ ABIN5596829) courtesy of MS Validated Antibodies.



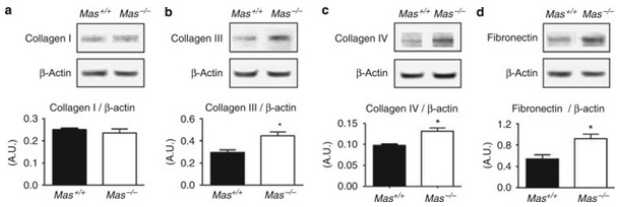
Immunohistochemistry
Immunohistochemistry results of Rabbit Anti-Collagen Type III Antibody. Tissue: human oral cavity. Fixation: FFPE. Antigen Retrieval: HIER using Tris-EDTA-citrate buffer pH 7.8 for 5 min. Blocking: Peroxidase-Blocking Solution for 10 min. Primary Antibody: Anti-Collagen Type III (p/n 600-401-105-0.1) at 1:15 for 1 hr at 37 °C. Secondary Antibody: Dako REAL EnVision Detection Kit, Polymer-HRP, Rabbit/Mouse. Counterstain: Hematoxylin for 15 sec. Substrate: DAB-Chromogen, Rabbit/Mouse. Staining/Results: Distinct fibrillar collagen III staining of the stroma in squamous cell carcinoma of the oral cavity. Independently Validated by antibodies-online GmbH (p/n ABIN7565873/ ABIN5596830/ ABIN5596829) courtesy of MS Validated Antibodies.



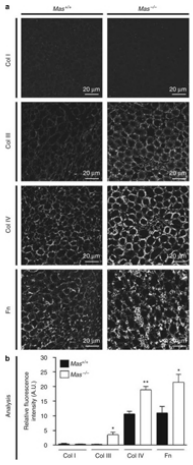
Immunohistochemistry
Immunohistochemistry results of Rabbit Anti-Collagen Type III Antibody. Tissue: human heart muscle. Fixation: FFPE. Antigen Retrieval: HIER using Tris-EDTA-citrate buffer pH 7.8 for 5 min. Blocking: Peroxidase-Blocking Solution for 10 min. Primary Antibody: Anti-Collagen Type III (p/n 600-401-105-0.1) at 1:15 for 1 hr at 37 °C. Secondary Antibody: Dako REAL EnVision Detection Kit, Polymer-HRP, Rabbit/Mouse. Counterstain: Hematoxylin for 15 sec. Substrate: DAB-Chromogen, Rabbit/Mouse. Staining/Results: Distinct fibrillar collagen III staining surrounding each heart muscle cell. Independently Validated by antibodies-online GmbH (p/n ABIN7565873/ABIN5596830/ABIN5596829) courtesy of MS Validated Antibodies.



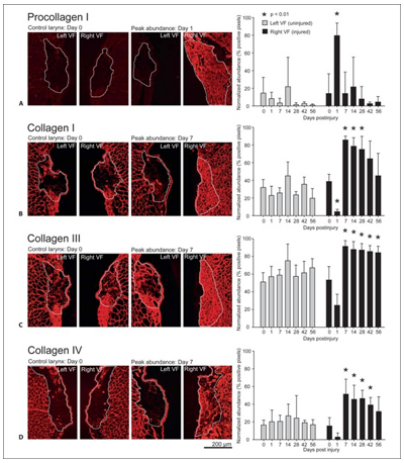
Immunohistochemistry
Immunohistochemistry results of Rabbit Anti-Collagen Type III Antibody. Tissue: human non-cancerous breast tissue. Fixation: FFPE. Antigen Retrieval: HIER using Tris-EDTA-citrate buffer pH 7.8 for 5 min. Blocking: Peroxidase-Blocking Solution for 10 min. Primary Antibody: Anti-Collagen Type III (p/n 600-401-105-0.1) at 1:225 for 1 hr at 37 °C. Secondary Antibody: Dako REAL EnVision Detection Kit, Polymer-HRP, Rabbit/Mouse. Counterstain: Hematoxylin for 15 sec. Substrate: DAB-Chromogen, Rabbit/Mouse. Staining/Results: Fibrillar collagen III staining in non-cancerous breast tissue showing considerable sclerosis. Independently Validated by antibodies-online GmbH (p/n ABIN7565873/ ABIN5596830/ ABIN5596829) courtesy of MS Validated Antibodies.



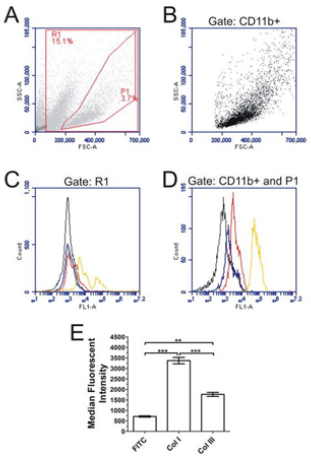
Western Blot
Immunoblotting of extracellular matrix (ECM) proteins in kidneys of Mas+/+ and Mas−/− animals. (a) Immunoblotting shows no difference of Collagen I (p/n 600-401-103) expression in Mas+/+ and Mas−/− mice kidneys. Significant increases in (b) Collagen III (p/n 600-401-105), (c) Collagen IV (p/n 600-401-106), and (d) fibronectin (p/n 600-401-117) expression were detected by comparing immunoblots of Mas−/− mouse kidneys with those of Mas+/+ controls. Each band represents one mouse kidney from either Mas+/+ or Mas−/− mice. Data are shown as the mean±s.e.m. *P<0.05. A.U. indicates arbitrary unit. Fig 5. PMID: 19262461



Immunofluorescence Microscopy
Immunofluorescence of extracellular matrix (ECM) proteins in the medulla of kidneys from Mas+/+ (left column) and Mas−/− (right column) mice. (a) Fluorescence (Cy3-labeled anti-rabbit IgG) reveals the immunolabeling of ECM proteins. Expression of type III collagen (Col III) (p/n 600-401-105), type IV collagen (Col IV) (p/n 600-401-106), and fibronectin (Fn) (p/n 600-401-117) were increased in the medulla of Mas−/− compared with that of Mas+/+ mice, whereas the expression of type I collagen (Col I) (p/n 600-401-103) was unaltered. (b) Quantification of ECM proteins in the medulla of Mas+/+ and Mas−/− mice. Data are shown as mean±s.e.m. *P<0.05; **P<0.01. A.U. indicates arbitrary unit. Fig 3. PMID: 19262461

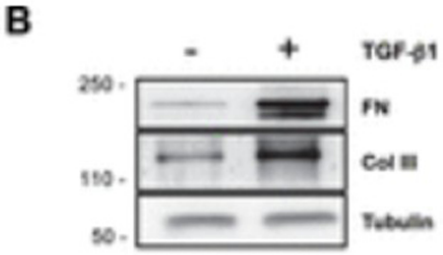


Immunohistochemistry
Representative IHC images and quantitative analysis of procollagen type I (A), collagen type I (B), collagen type III (C), and collagen type IV (D) in uninjured control and injured vocal fold LP over time. Normalized abundance is expressed with respect to total LP area (indicated by a white dashed line). Error bars represent standard deviations and asterisks denote statistically significant differences ($p < 0.01$) from the control group (day 0). VF = Vocal fold. Scale bar: 200 μm. Fig 2. PMID: 20516667



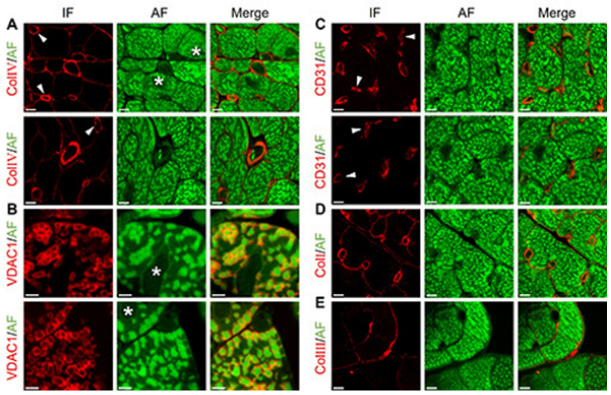
Flow Cytometry

ACK-treated spleen cells were cultured for 5 days at 3.5 × 105 cells per well in the presence of IL-13 and M-CSF. Adherent cells were removed by trypsin-EDTA treatment and stained for the presence of CD11b. The cells were then fixed, permeabilized, stained with rabbit polyclonal antibodies, and analyzed by flow cytometry. A) Forward and side scatter characteristics of 5 day cultured spleen cells. (B) Forward and side scatter analysis of CD11b+ cells. C and D) Histograms show fluorescence intensity of FITC-conjugated goat F(ab')2 anti-rabbit 2° (black line) compared to collagen I (red line), collagen III (blue line), and as a positive control syk (yellow line). Flow cytometry plots are representative of 3 independent experiments. C) When gating the entire live cell population R1 (from A), collagen was nearly undetectable, but when gating (D) for CD11b+ cells and region P1 (from A and B) there was an increase in the levels of both collagen I and III. E) Compared to FITC control, CD11b+ cells had a significant increase in median fluorescent intensity for collagen I and III staining (1-way ANOVA, Dunnett's test). **, $p < 0.01$; ***, $p < 0.001$. Fig 2. PMID: 20888336

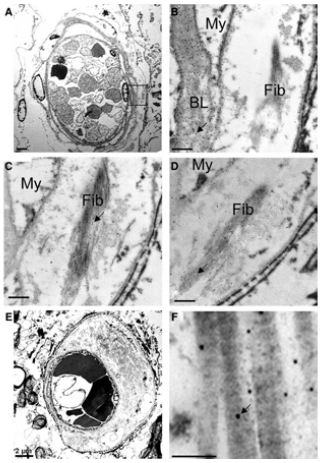


Western Blot

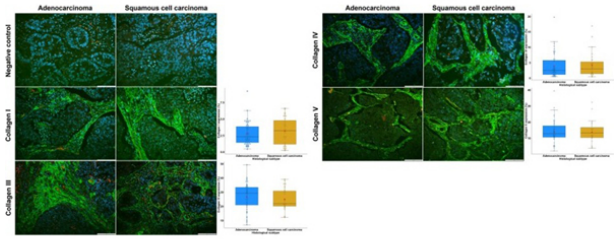
B: representative Western blot of fibronectin (FN) and collagen type III (Col III) levels in C3H 10T1/2 fibroblasts treated with TGF-β1 5 ng/ml. Tubulin was used as the loading control. Fig 3. PMID: 29351412



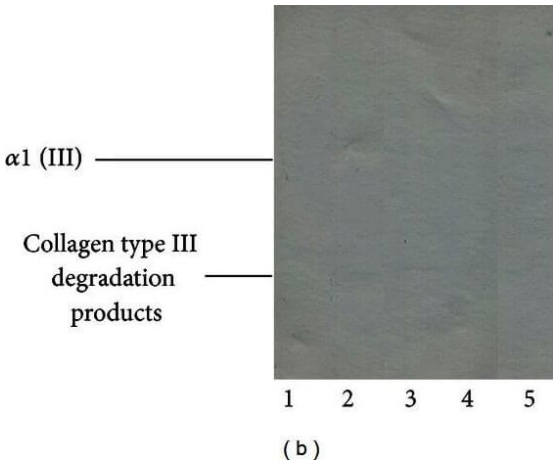
Immunofluorescence Microscopy
Major autofluorescent structures in formaldehyde-fixed mouse myocardial samples identified by IF stainings of (A) Col IV, (B) VDAC1, (C) CD31, (D) Col I (p/n 600-401-103, and (E) Col III (p/n 600-401-105). Images were acquired at cardiomyocyte cross-sectional areas. IF staining (red) allows the localization of basement membrane (Col IV), mitochondria (VDAC1), microvascular endothelial cells (CD31), and interstitial collagen network (Col I and Col III), facilitating the identification of observable structures in AF images (green) acquired at CH1 (525±25 nm). Oval-to-rectangular nuclei (star signs) are observed within cardiomyocytes, and erythrocytes are present within some capillaries (arrowheads). Scale bars: A,C-E) 5 µm; B) 2 µm. Fig 3.
PMID: 37781779



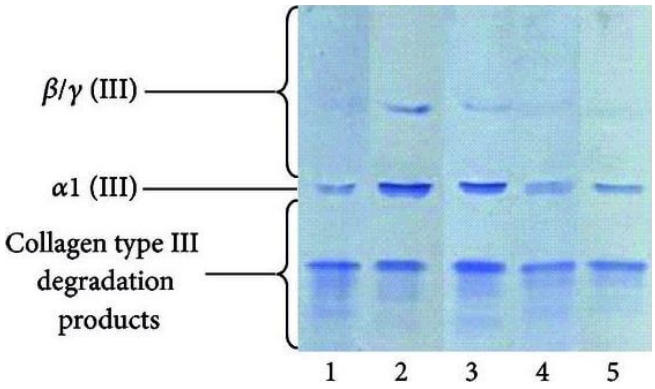
Immunofluorescence Microscopy
Immuno-electron microscopy of collagens I, III, and IV in small vessel disease (SVD). Transmission electron micrographs of small arteries in white matter from older persons with SVD. A–D, Semi-serial sections of a small arteriole, shown at low magnification in A and at higher magnification in B–D with immunogold labeling for collagen-IV (p/n 600-401-106) (B), collagen-I (p/n 600-401-103) (C), or collagen-III (p/n 600-401-105) (D). The region shown at higher magnification in B–D is marked in A (box). Arrows show examples of immunogold particles. In B, collagen-IV labeling shows numerous gold particles over the homogenous basal laminae (BL), behind the endothelium. Mural collagen fibrils (Fib) and myocytes (My) are unlabeled. C, Collagen-I, in contrast to collagen-IV, is mainly localized to the collagen fibrils. D, Collagen-III also labels the banded collagen fibrils. A few gold particles are found over the basal laminae. E and F, Another small artery with severe fibrosis. Low magnification view (E) shows heavy deposits of fibrillar collagens in the vessel wall. In higher magnification, these are heavily labeled for collagen-I (F). Scale bars: 2 µm (A and E), 0.5 µm (B–D), and 0.2 µm (F). Fig 3.
PMID: 36205142



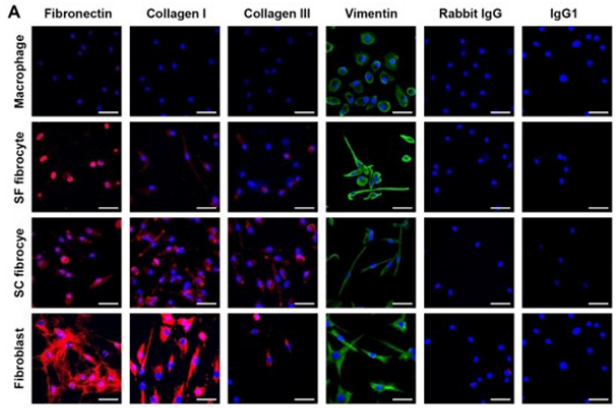
Immunofluorescence Microscopy
Immunofluorescence expression of collagens in the extracellular matrix in lung adenocarcinoma and lung squamous cell carcinoma. The panel shows the negative control, collagen I, collagen III, collagen IV and collagen V. Also as presented the respectively box-plots show the differences of expression between the histological subtypes. The red square in the graphs represents the average expression of the marker for each of the groups. Magnification: 400×. Scale: 100 µm. Fig. 2. PMID: 39938232



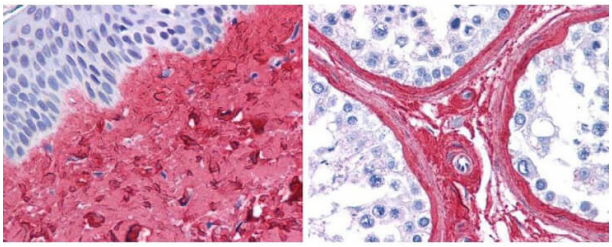
Western Blot
(a) Typical electropherogram of α (I) subunits, oligomers β/γ (I), and collagen type I degradation products, released from healthy and burnt skin. Collagenous components released from tissue samples using pepsin were submitted to 4–15% gradient SDS-PAGE, in nonreducing conditions. (b) Interferences of collagen type III, in electrophoretic profiles of collagen type I components extracted from healthy and burned skin. Collagen components were submitted to electrophoresis in the absence of dithiothreitol (reducing disulfide bonds) and subsequently—after electrotransfer to Immobilon—subjected to reaction with collagen type III antibodies. Lane 1: components of collagen type I isolated from healthy skin. Lane 2: components of collagen type I isolated from burned skin treated with propolis. Lane 3: components of collagen type I isolated from burned skin treated with AgSD. Lane 4: components of collagen type I isolated from burned skin treated with propolis vehicle. Lane 5: components of collagen type I isolated from burned skin treated with NaCl. Figure provided by CiteAb. Source: Evid Based Complement Alternat Med, PMID: 23781260.



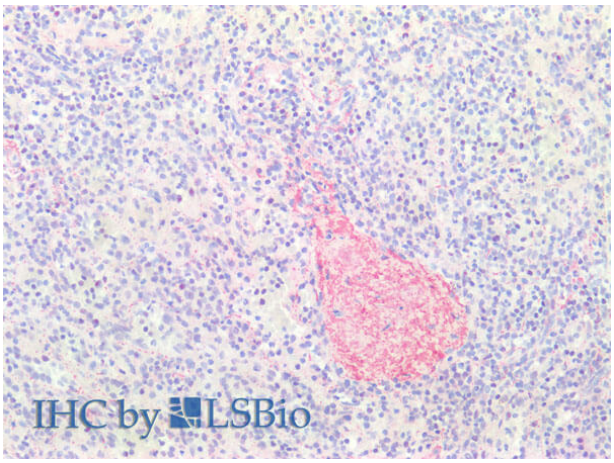
Western Blot
Typical electropherogram of α (III) subunits, oligomers β/γ (III), and collagen type III degradation products, released from healthy and burned skin. Collagenous components released from tissue samples using pepsin were submitted to 4–15% gradient SDS-PAGE, western blotted, and probed with collagen type III antibodies. Lane 1: components of collagen type I isolated from healthy skin. Lane 2: components of collagen type I isolated from burned skin treated with propolis. Lane 3: components of collagen type I isolated from burned skin treated with AgSD. Lane 4: components of collagen type I isolated from burned skin treated with propolis vehicle. Lane 5: components of collagen type I isolated from burned skin treated with NaCl. Figure provided by CiteAb. Source: Evid Based Complement Alternat Med, PMID: 23781260.



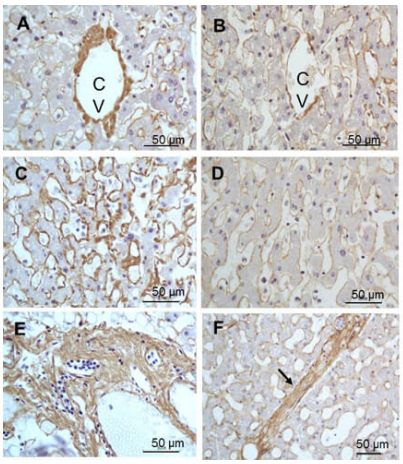
Immunofluorescence Microscopy
Fibrocytes were generated from PBMC under serum-free (SF) and serum-containing (SC) culture conditions, as well as macrophages. Fibroblast cell lines were included as a positive control for stromal cell markers. Immunostaining is shown in red for the stromal markers fibronectin, collagens I and III and their rabbit IgG control, with vimentin and its mouse IgG1 control in green (A). Nuclear staining is shown in blue. Bar represents 50 µm. Data are representative of at least two separate experiments. Fig 2. PMID: 20305780



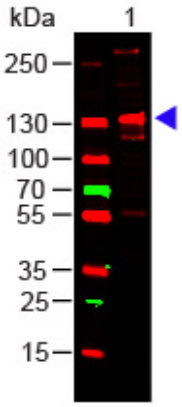
Immunohistochemistry
Rockland anti collagen III antibody (600-401-105 Lot 26016, 1:400, 45 min RT) showed strong staining in FFPE sections of human skin(left, dermis) with moderate to strong red staining and testis (right) where strong staining was observed within connective tissue between seminiferous tubules. The antibody showed strong extracellular staining within connective tissues across many organs with minimal background staining. Slides were steamed in 0.01 M sodium citrate buffer, pH 6.0 at 99-100°C - 20 minutes for antigen retrieval. Images provided courtesy of LifeSpan Biosciences, Seattle, WA



Immunohistochemistry
Immunohistochemistry of Rabbit Anti-Collagen Type III Antibody. Tissue: FFPE normal human spleen tissues (10X). Antigen Retrieval: 0.01 M sodium citrate buffer for 20 minutes. Primary Antibody: Anti-Collagen Type III at 5µL/mL for 45 mins at RT. Staining: Anti-Rabbit biotinylated secondary antibody for 30 min at RT. Alkaline phosphatase streptavidin for 30 min at RT. Alkaline phosphatase chromogen substrate for 30 min at RT. The stained slides were evaluated by a pathologist to confirm staining specificity.



Immunohistochemistry
Immunohistochemistry of Rabbit Anti-collagen type III antibody. Tissue: right lobe of the liver section. A:Central Vein (CV) fibrosis, B: Non-fibrotic CV, C: Perisinusodial fibrosis, D: Non-fibrotic area, E: Protat tract fibrosis, F: Septal fibrosis (arrow). Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: Anti-collagen type III at 1:500 for 4°C for 24hr. Secondary antibody: Peroxidase biotin-streptavidin rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: Anti-collagen type III is intra and extracellular. Staining: 3.3'-diaminobenzidine tetrahydrochloride was used as the chromogen. Nuclei were counterstained purple with hematoxylin.



Western Blot
Western Blot of Rabbit Anti-COLLAGEN III Antibody. Lane 1: Human Collagen III (p/n 009-001-105). Load: 100ng per lane. Primary antibody: Collagen III Antibody at 1:1000 o/n at 4°C. Secondary antibody: DyLight™ 649 Goat anti-rabbit (p/n 611 -1302) at 1:20,000 for 30 min at RT. Block: MB-070 for 30 min at RT. Predicted/Observed size: 138 kDa, 138 kDa.

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