Datasheet for ABIN5596835

anti-Collagen IV antibody

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Overview

Quantity:	100 µg
Target:	Collagen IV (COL4)
Reactivity:	Human, Cow
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This Collagen IV antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunohistochemistry (IHC), Immunoprecipitation (IP), Dot Blot (DB), Fluorescence Microscopy (FM)

Product Details

Purpose:	Collagen Type IV Antibody
Immunogen:	Immunogen: Collagen Type IV from human and bovine placenta Immunogen Type: Native Protein
Isotype:	IgG
Cross-Reactivity (Details):	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.
Characteristics:	Synonyms: rabbit anti-Collagen Type IV antibody, Arresten antibody, Canstatin antibody, Collagen Of Basement Membrane Alpha 1 Chain antibody, Collagen alpha-1 (IV) chain, COL4A1
Purification:	Anti-Collagen Type IV has been prepared by immunoaffinity chromatography using immobilized antigens followed by extensive cross-adsorption against other collagens, human serum

Product Details

	proteins and non-collagen extracellular matrix proteins to remove any unwanted specificities.
Sterility:	Sterile filtered

Target Details

Target:	Collagen IV (COL4)
Alternative Name:	Collagen Type IV (COL4 Products)
Background:	Background: 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.
Gene ID:	1282
NCBI Accession:	NP_001290039
UniProt:	P02462

Application Details

Application Notes:	Immunohistochemistry Dilution: 1:50 - 1:200 Application Note: Anti-Collagen Type IV has been tested by dot blot and IHC and is suitable 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. Western Blot Dilution: 1:1,000 - 1:10,000 Immunoprecipitation Dilution: 1:100 ELISA Dilution: 1:5,000 - 1:50,000 IF Microscopy Dilution: User Optimized Other: User Optimized

Application Details

Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1.0 mg/mL
Buffer:	Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 Stabilizer: None Preservative: 0.01 % (w/v) Sodium Azide
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C,-20 °C
Storage Comment:	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.
Expiry Date:	12 months

Publications

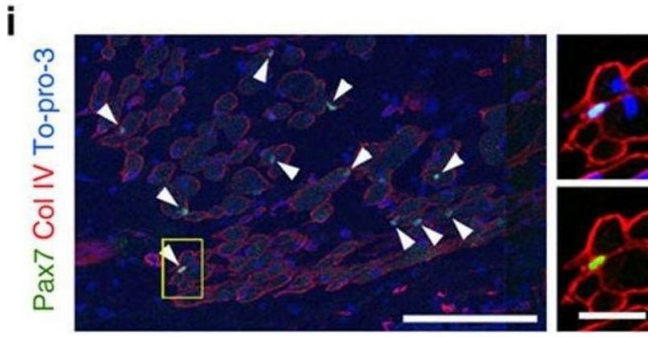
Product cited in:	Ida, Hikage, Itoh, Ida, Ohguro: "Prostaglandin F2α agonist-induced suppression of 3T3-L1 cell adipogenesis affects spatial formation of extra-cellular matrix." in: **Scientific reports**, Vol. 10, Issue 1, pp. 7958, (2020) (PubMed). Kumar Gupta, Sarkar, Wertheim, Pan, Carroll, Oxburgh: "Asynchronous mixing of kidney progenitor cells potentiates nephrogenesis in organoids." in: **Communications biology**, Vol. 3, Issue 1, pp. 231, (2020) (PubMed). Hwang, Huang, Burwell, Peterson, Connor, Weiss, Yu, Li: "In Situ Imaging of Tissue Remodeling with Collagen Hybridizing Peptides." in: **ACS nano**, Vol. 11, Issue 10, pp. 9825-9835, (2019) (PubMed). Tanaka, Ng, Yang Yu, Casco-Robles, Maruo, Tsonis, Chiba: "A developmentally regulated switch from stem cells to dedifferentiation for limb muscle regeneration in newts." in: **Nature**

communications, Vol. 7, pp. 11069, (2016) (PubMed).

Fetting, Guay, Karolak, Iozzo, Adams, Maridas, Brown, Oxburgh: "FOXD1 promotes nephron progenitor differentiation by repressing decorin in the embryonic kidney." in: **Development (Cambridge, England)**, Vol. 141, Issue 1, pp. 17-27, (2014) (PubMed).

There are more publications referencing this product on: Product page

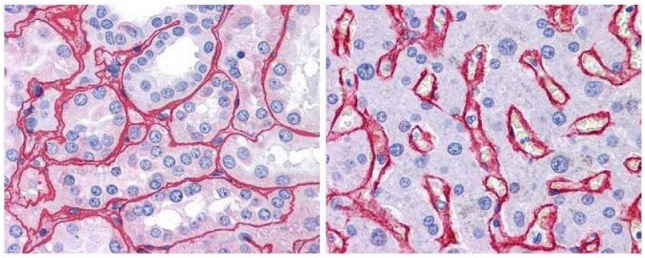
Images



Immunofluorescence (Paraffin-embedded Sections)

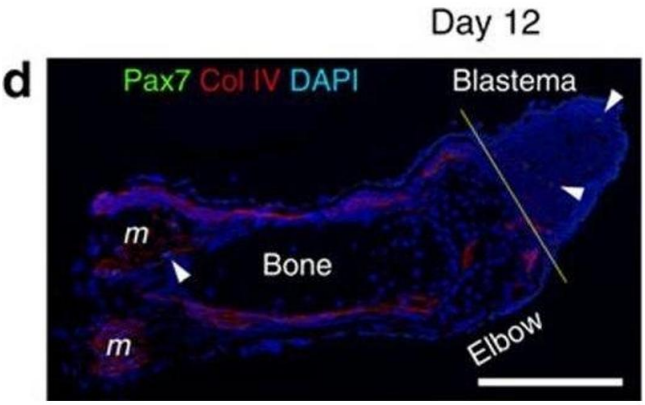
Image 1. SMFC tracking in metamorphosed newt limb regeneration.(a) Juvenile (16 months old). Scale bar, 15mm. (b) Limb regeneration. Scale bar, 5mm. (c-e) Tracking of SMFCs (mCherry+) (n=2). This animal was a mosaic expressing EGFP in muscle only. mCherry+ fibres in the forearm were ~25 % of total EGFP+ fibres. (c) On day 36 after amputation, fragments of muscle fibres (arrows) were observed in distal regions adjacent to the blastema. Scale bar, 100µm. (d) mCherry+ mononucleated cells (red arrowheads, enlarged in right-hand panels) and EGFP+ cells (green arrowheads) were observed in the blastema. Epi, epidermis. To-pro-3: nuclei. Scale bars, 50µm (left), 10µm (right-hand panels). (e) In the same limb, at day 96 after the second amputation in the upper arm (line), mCherry (arrows) and EGFP were observed only in muscle fibres. Scale bars, 1mm (upper panel), 500µm (lower panels). (f-i) Pax7 immunolabelling of a regenerating limb on day 26 after amputation (n=4). Pax7 immunoreactivity was not detected in the blastema. (f) Translucent image. Line: amputation site. (g) Merged fluorescence image. Col IV, collagen type IV immunoreactivity. To-pro-3: nuclei. Scale bar, 1mm. (h) Enlargement of a region in the blastema and (i) a region proximal to the amputation site, enclosed by boxes in g. Scale bars, 250µm. Arrowheads in (i) Pax7+

nuclei. An example satellite cell (box) is enlarged in the right-hand panels (upper: Col VI/To-pro-3, lower: Col IV/Pax7). Scale bar, 50μm. (j) Summary. In metamorphosed newts, SMFCs were recruited for new muscle during limb regeneration, whereas MPCs such as satellite cells were not. - figure provided by CiteAb. Source: PMID27026263



Immunohistochemistry

Image 2. anti collagen IV antibody (600-401-106 Lot 25440, 1:400, 45 min RT) showed strong staining in FFPE sections of human kidney (Left) with strong red staining observed in glomeruli; and liver (Right) with strong staining in sinusoids. Staining for both tissues was consistent with a basement membrane distribution. 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



Immunofluorescence (Paraffin-embedded Sections)

Image 3. SMFC tracking in larval newt limb regeneration.(a) Larva (3 months old). It has four limbs, as well as the gills and tail fin. Scale bar, 4mm. (b) Monitoring of SMFCs (mCherry+) during limb regeneration (n=6). mCherry was not detected in the regenerating part of the limb until ~30 days when the amputated limb had almost been recovered (see Supplementary Movie 1). Arrowheads: flexor muscle for digits (see Fig. 2). Scale bar, 1mm. (c) Sections of regenerating limbs (n=3 for each stage). SMFC-derived mCherry+ cells were not observed in the blastema. Lines: amputation site. m: muscle. Scale bar, 100μm. (d-f) Pax7 immunolabelling of regenerating limbs on day 12 (n=3) and (g) day 15 (n=3) after amputation. (d) On day 12, a few Pax7+ nuclei (arrowheads) were detected in blastema cells and in satellite cells along the muscle fibres. Col IV, collagen type IV immunoreactivity. DAPI (4,6-diamidino-2-phenylindole), nuclei. Scale bar, 300μm. The Pax7+ nuclei

pointed by arrowheads were enlarged in e and f, respectively. Scale bars, 100μm. (g) On day 15 when the regenerating part of the limb grew more distally, the number of Pax7+ nuclei (arrowheads) in the blastema was dramatically increased. Scale bar, 100μm. (h) Summary. In larval newts, MPCs, potentially satellite cells, were recruited for new muscle during limb regeneration, whereas SMFCs were not. - figure provided by CiteAb. Source: PMID27026263

Please check the product details page for more images. Overall 9 images are available for ABIN5596835.

Successfully validated (Immunohistochemistry (IHC))

by MS Validated Antibodies

Report Number: 300049

Date: Aug 07 2023

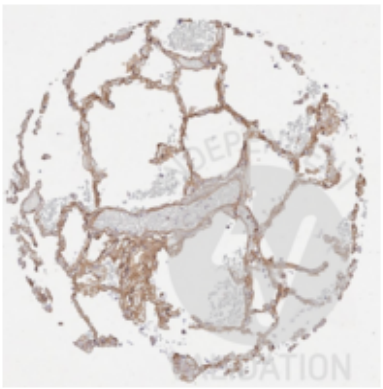
Target:	COL4
Lot Number:	48011
Method validated:	Immunohistochemistry (IHC)
Positive Control:	Human TMA Recombinant rabbit anti-COL4 antibody (MSVA-704R)
Notes:	Passed. Staining of collagen type IV using ABIN5596835 is consistent with the expected staining pattern.
Primary Antibody:	ABIN5596835
Secondary Antibody:	EnVision Polymer-HRP mouse/rabbit Kit, Dako REAL, K5007
Protocol:	• Slide preparation ◦ Mount 2,5 µm FFPE tissue sections on superfrost slides. ◦ Deparaffinize tissue sections 3x 5 min in xylene. ◦ Rehydrate tissue sections in a descending ethanol series for 1 min each 100%, 96%, and 80% ethanol. ◦ Rinse tissue sections for 5 min in TBST buffer (DAKO, K8000). • Epitope retrieval ◦ Autoclave tissue sections for 5 min at 121 °C in 1x Tris-EDTA-citrate buffer pH7.8 (20x Tris-EDTA-citrate buffer stock solution: 5 g Trizma base (Sigma-Aldrich, T1503), 10 g EDTA (Merck, 1.08418), 6.4g tri-sodium citrate (Sigma-Aldrich, C0909), adjust to pH 7.8 using HCL 1 M, ad 1 L with dH_2O). ◦ Rinse tissue sections for 5 min in TBST buffer. • Peroxidase blocking ◦ Incubate tissue sections for 10 min in Peroxidase-Blocking Solution (Dako REAL, S2023). ◦ Rinse tissue sections 2x for 5 min in TBST buffer. • Antibody incubation ◦ Dilute primary rabbit anti-collagen IV antibody (antibodies-online, ABIN5596835, lot 48011) diluted 1:100, 1:200, or 1:300 in antibody diluent (Dako REAL, S2022). ◦ Cover tissue section with 100-200 µl diluted antibody. ◦ Incubate tissue sections for 1 h at 37 °C in a moist chamber. ◦ Rinse tissue sections for 5 min in TBST buffer.

- Apply EnVision Polymer-HRP mouse/rabbit Kit (Dako REAL, K5007) according to manufacturer's recommendation.
- Rinse tissue sections 2x for 5 min in TBST buffer.

- Staining
  - Cover slides for 10 min with DAB-Chromogen (EnVision Polymer-HRP mouse/rabbit Kit, Dako REAL, K5007).
  - Wash slides thoroughly with dH_2O.
  - Counterstain for 15 sec with Hematoxylin (Mayers Hematoxylin: 200ml ddH_2O, 0,2g Hematoxylin (Serva, 24420.02), 10 g aluminium potassium sulfate dodecahydrate (Merck, 1.01047), 0,04 g sodium iodate (Merck, 1.06525), 10 g chloral hydrate (Sigma-Aldrich, 15307)).
  - Develop for 15 sec in H_2O.
  - Dehydrate tissue sections in an ascending ethanol series for 1 min each 80%, 96%, 100% ethanol.
  - Wash tissue sectiona 3x 5 min in xylene.
  - Apply mounting medium and coverslips.
- Image acquisition
  - o Acquire images using a Galileo TMAtic (ISENET).

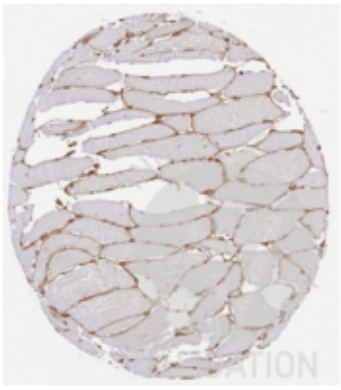
Experimental Notes:

- For antibody comparison an antibody test TMA was used that contained 80 normal tissues from 21 different organs and 95 neoplastic tissues from 18 different tumor types. Despite some cytoplasmic and stromal background ABIN5596819 identified identical structures as the reference antibody MSVA-704R. An additional occasional staining of nuclei which was only seen by ABIN5596819 must be considered a cross-reactivity.
- According to the rather ubiquitous nature of collagen IV expression, orthogonal validation is not optimally suited for the validation of collagen I antibodies. In agreement with RNA screening studies, including the Human Protein Atlas (HPA) RNA-seq tissue dataset, the FANTOM5 project, and the Genotype-Tissue Expression (GTEx) project (all summarized in https://www.proteinatlas.org/ENSG00000134871-COL4A2/tissue), collagen IV staining by ABIN5596819 was particularly high in the placenta, colon, smooth muscle, and the lung. These are among the tissues with the highest recorded RNA expression.



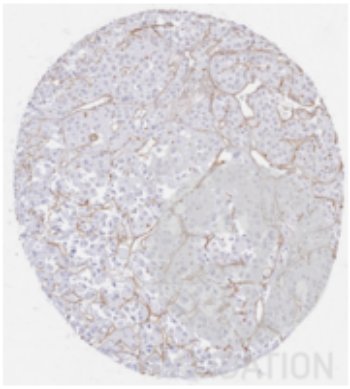
Validation image no. 1 for anti-Collagen, Type IV (COL4) antibody (ABIN5596835)

IHC staining of lung tissue with anti-collagen IV antibody ABIN5596835 diluted 1:200 shows staining of basement membranes and vessels.



Validation image no. 2 for anti-Collagen, Type IV (COL4) antibody (ABIN5596835)

IHC staining skeletal muscle tissue with anti-collagen IV antibody ABIN5596835 diluted 1:200 shows collagen IV fibres surrounding skeletal muscle cells.



Validation image no. 3 for anti-Collagen, Type IV (COL4) antibody (ABIN5596835)

IHC staining of renal oncocytoma tissue with anti-collagen IV antibody ABIN5596835 diluted 1:300 shows dense collagen IV-positive membranes surrounding tumor cell nests.