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Polyclonal Antibody to Collagen type I - Purified

Alternate names: Alpha-1 type I collagen, Alpha-2 type I collagen, COL1A1, COL1A2

Catalog No.: R1038X

Quantity: 0.5 mg

Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

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. Collagens for immunization from human and bovine placenta and cartilage have been extensively purified 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.

Uniprot ID: P02452

NCBI: NP_000079.2

GeneID: 1277

Host: Rabbit

Immunogen: Collagen type I purified from Human and Bovine placenta.

Format:
State: Liquid (sterile filtered) purified Ig fraction.
Purification: Immunoaffinity Chromatography.
Buffer System: 0.1M Sodium Borate, 0.075M Sodium Chloride, 0.005M EDTA, pH 8.0
Preservatives: 0.01% (w/v) Sodium Azide
Stabilizers: None

Applications: Anti-Collagen antibodies have been used for indirect trapping ELISA for quantitation of antigen in serum using a standard curve, for Immunoprecipitation and for native (non-denaturing, non-dissociating) PAGE and western blotting for highly sensitive qualitative analysis. Specific researchers have reported that this antibody is also functional by conventional SDS-PAGE western blot. See references below for additional details.

Recommended Dilutions:
ELISA: 1/5,000-1/50,000.
Western blot: 1/1,000-1/10,000.
Immunoprecipitation: 1/100.
Immunohistochemistry: 1/50-1/200.

Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:

This product has been prepared by Immunoaffinity Chromatography using immobilized antigens followed by extensive cross-adsorption against other collagens, human serum proteins and non-collagen extracellular matrix proteins to remove any unwanted specificities.
Typically less than 1% cross reactivity against other types of collagens was detected by ELISA against purified standards. 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 I Collagens and has negligible cross-reactivity with Type II, III, IV, V or VI collagens. Non-specific cross-reaction of anti-collagen antibodies with other Human serum proteins or non-collagen extracellular matrix proteins is negligible.
Species: Human, Mouse, Rat and Bovine.
Other species not tested.

Storage:

Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
For extended storage, mix with an equal volume of glycerol.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

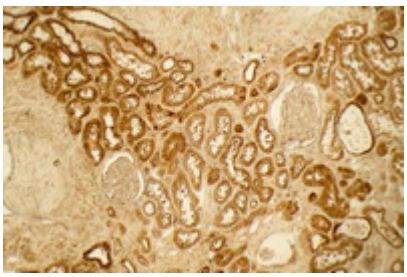
Product Citation:

1. Daniela Villone, Anja Fritsch, Manuel Koch, Leena Bruckner-Tuderman, Uwe Hansen, and Peter Bruckner Supramolecular Interactions in the Dermo-epidermal Junction Zone: ANCHORING FIBRIL-COLLAGEN VII TIGHTLY BINDS TO BANDED COLLAGEN FIBRILS. *J. Biol. Chem., Sep* ***2008****; 283: 24506-24513.*
2. Daniel Timo Behrens, Daniela Villone, Manuel Koch, Georg Brunner, Lydia Sorokin, Horst Robenek, Leena Bruckner-Tuderman, Peter Bruckner, and Uwe Hansen: The Epidermal Basement Membrane Is a Composite of Separate Laminin- or Collagen IV-containing Networks Connected by Aggregated Perlecan, but Not by Nidogens. *J. Biol. Chem.,* May **2012**; 287: 18700-18709.

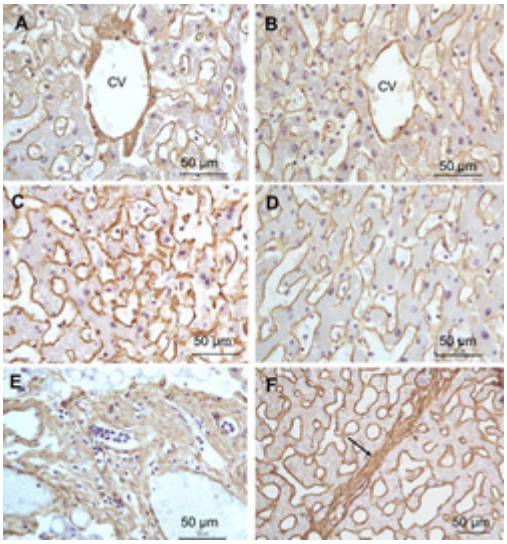
General References:

1. Stefanovic, B, Schnabl, B, Brenner, DA (2002) Inhibition of collagen alpha 1(I) expression by the 5' stem-loop as a molecular decoy. *J.Biol.Chem.* 277(20):18229-18237.
2. Hashimoto, N et al. (2004) Bone marrow derived progenitor cells in pulmonary fibrosis. *J. Clin. Invest.* 113:243-252.
3. Hazra, S et al. (2004) Peroxisome Proliferator-activated Receptor γ Induces a Phenotypic Switch from Activated to Quiescent Hepatic Stellate. *Cells. J. Biol. Chem.* 279(12):11392-11401.
4. She, H, Xiong, S, Hazra, S, Tsukamoto, H (2004) Adipogenic transcriptional regulation of hepatic stellate cells. *JBC* Papers in Press. Published on November 9, 2004 as Manuscript M410078200.

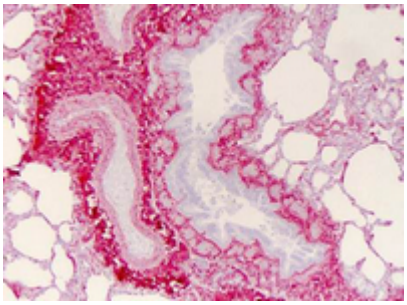
Pictures:



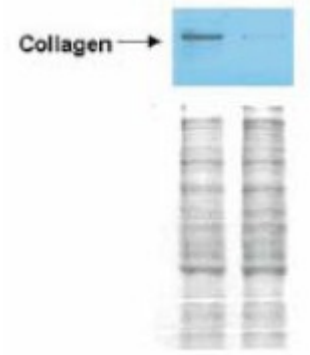
Immunohistochemistry using affinity purified Collagen type I antibody Cat.-No, R1038 at a 1:100 dilution to detect distal tubules in normal kidney tissue. Note the absence of staining of glomeruli. The antibody was reacted with antibody for 4 h RT followed by secondary antibody and substrate reaction. Tissue was Formalin-fixed and Paraffin embedded. No antigen retrieval was performed.



Immunohistochemistry of a liver section (Formalin-fixed, Paraffin-embedded) using Collagen type I antibody Cat.-No. R1038. A:Central vein (CV) fibrosis, B: Non-fibrotic CV, C: Perisinusodial fibrosis, D: Non-fibrotic area, E: Protat tract fibrosis, F: Septal fibrosis (arrow). Primary antibody: Collagen type I antibody at 1:1250 for 4°C for 24hr; Secondary antibody: Peroxidase biotin-streptavidin rabbit secondary antibody at 1:10,000 for 45 min at RT; Localization: Collagen type I is intra- and extracellular; Staining: 3.3'-diaminobenzidine tetrahydrochloride was used as the chromogen. Nuclei were counterstained purple with hematoxylin.



Immunohistochemistry of human lung tissue (Formalin-fixed, Paraffin-embedded) using Collagen type I antibody Cat.-No. R1038: Primary antibody (Collagen I) at 1:400, secondary antibody: Peroxidase goat anti-rabbit at 1:10,000 for 45 min at RT; Localization: Strong staining was observed in the extracellular matrix of the lung. Epithelial cells were negative; Staining: Antibody as precipitated red signal with a hematoxylin purple nuclear counterstain.



Western blot analysis is shown using Collagen type I antibody Cat.-No. R1038 to detect expression of collagen I in Wistar rat hepatic stellate cells (HSC) in control (GFP-transduced) (left lane) and PPARgamma-transduced cell lysates (right lane). Protein staining shown below each blot depicts equal protein loading. An equal amount of the whole cell protein (100 µg) was separated by SDS-PAGE and electroblotted to nitrocellulose membranes. Proteins were detected by incubating the membrane with Collagen type I antibody at a concentration of 0.2-2 µg/10 ml in TBS (100 mM Tris-HCl, 0.15 M NaCl, pH 7.4) with 5% Non-fat milk. Detection occurred by incubation with a horseradish peroxidase-conjugated secondary antibody at 1 µg/10 ml. Proteins were detected by a chemiluminescent method using the PIERCE ECL kit (Amersham Biosciences). Other detection systems will yield similar results. See Hazra et al. (2004) for additional details.