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Schillerstr. 5

Polyclonal Antibody to Collagen type I - Purified R1038

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

Quantity: 0.1 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: 9606 GeneID: 1277 Rabbit Host:

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

Genename: COL1A1

Format: State: Liquid (sterile filtered) purified Ig fraction

Purification: Immunoaffinity Chromatography

Buffer System: 0.125M 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.



Molecular Weight:

150 kDa (Target)

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 crossreactivity with Type II, III, IV, V or VI collagens. Non-specific cross-reaction of anticollagen antibodies with other Human serum proteins or non-collagen extracellular matrix proteins is negligible.

Species: Human, Mouse, Rat and Bovine.

Other species not tested.

Add. Information:

Note: Collagen type I consists of alpha-1 (139 kDa) and alpha-2 chains (129kDa). Since collagen type I is a triple helix consisting of one alpha-2 chain and two alpha-1 chains, one can expect bands of the dimeric (~270 kDa) and the trimeric form (~400 kDa). Remember that those chains are cross-linked and can't be broken by typical sample denaturation for SDS-PAGE.

Storage:

Store 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 Citations:

Purchased from Acris:

- 1. Villone D, Fritsch A, Koch M, Bruckner-Tuderman L, Hansen U, Bruckner P. Supramolecular interactions in the dermo-epidermal junction zone: anchoring fibrilcollagen VII tightly binds to banded collagen fibrils. J Biol Chem. 2008 Sep 5;283(36):24506-13. doi: 10.1074/jbc.M802415200. Epub 2008 Jul 3. PubMed PMID: 18599485.
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- 3. Hoyas Fernández, JA. Terapia regenerativa del tendón supraespinoso: estudio realizado en un modelo murino de lesión crónica, Thesis 2014.
- 4. González-Miguel J, Morchón R, Siles-Lucas M, Simón F. Fibrinolysis and proliferative endarteritis: two related processes in chronic infections? The model of the blood-borne pathogen Dirofilaria immitis. PLoS One. 2015 Apr 13;10(4):e0124445. doi: 10.1371/journal.pone.0124445. eCollection 2015. PubMed PMID: 25875022. 5. Nyström A, Thriene K, Mittapalli V, Kern JS, Kiritsi D, Dengjel J, et al. Losartan ameliorates dystrophic epidermolysis bullosa and uncovers new disease

mechanisms. EMBO Mol Med. 2015 Jul 20;7(9):1211-28. doi: 10.15252/emmm.201505061. PubMed PMID: 26194911.



- 6. Carriel V, Scionti G, Campos F, Roda O, Castro B, Cornelissen M, et al. In vitro characterization of a nanostructured fibrin agarose bio-artificial nerve substitute. J Tissue Eng Regen Med. 2015 Jul 14. doi: 10.1002/term.2039. PubMed PMID: 26177604. 7. Caley MP, King H, Shah N, Wang K, Rodriguez-Teja M, Gronau JH, et al. Tumorassociated Endo180 requires stromal-derived LOX to promote metastatic prostate cancer cell migration on human ECM surfaces. Clin Exp Metastasis. 2016 Feb;33(2):151-65. doi: 10.1007/s10585-015-9765-7. Epub 2015 Nov 13. PubMed PMID: 26567111.
- 8. Oleg Tsuprykov2, Ryotaro Ando, Christoph Reichetzeder, Karoline von Websky, Viktoriia Antonenko, Yuliya Sharkovska, Lyubov Chaykovska, Jan Rahnenführer, Ahmed A. Hasan, Harald Tammen, Markus Alter, Thomas Klein, Seiji Ueda, Sho-ichi Yamagishi, Seiya Okuda, Berthold Hocher. The dipeptidyl peptidase inhibitor linagliptin and the angiotensin II receptor blocker telmisartan show renal benefit by different pathways in rats with 5/6 nephrectomy. Kidney International Volume 89, Issue 5, May 2016, Pages 1049–1061. doi:10.1016/j.kint.2016.01.016.
- 9. Confalonieri, D; Marca, ML; van Dongen, EM; Walles, H; Ehlicke, F. A Novel Injectable Recombinant Collagen I Peptide – based Macroporous Microcarrier Allows Superior Expansion of C2C12 and Human Bone Marrow - derived Mesenchymal Stromal Cells and Supports Deposition of Mineralized MatrixTissue Engineering Part A 2017. http://online.liebertpub.com/doi/abs/10.1089/ten.TEA.2016.0436.
- 10. Malischewski, A; Moreira, R; Hurtado, L; Gesché, V; Schmitz-Rode, T; Jockenhoevel, S; Mela, P. Umbilical cord as human cell source for mitral valve tissue engineering venous vs. arterial cellsBiomed Tech (Berl). PubMed PMID: 28453437.
- 11. Durand-Herrera, D; Campos, F; Jaimes-Parra, BD; Sánchez-López, JD; Fernández-Valadés, R; Alaminos, M; Campos, A; Carriel, V. Wharton's jelly-derived mesenchymal cells as a new source for the generation of microtissues for tissue engineering applicationsHistochem. Cell Biol. 2018. PubMed PMID: 29931444:
- 12: Godoy-Guzmán, C; Nuñez, C; Orihuela, P; Campos, A; Carriel, V: Distribution of extracellular matrix molecules in human uterine tubes during the menstrual cycle: a histological and immunohistochemical analysis: J. Anat. 2018. PubMed PMID: 29663371.

General Readings:

- 1. Stefanovic B, Schnabl B, Brenner DA. Inhibition of collagen alpha 1(1) expression by the 5' stem-loop as a molecular decoy. J Biol Chem. 2002 May 17;277(20):18229-37. Epub 2002 Mar 11. PubMed PMID: 11889120.
- 2. Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. J Clin Invest. 2004 Jan;113(2):243-52. PubMed PMID: 14722616.
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- 4. She H, Xiong S, Hazra S, Tsukamoto H. Adipogenic transcriptional regulation of hepatic stellate cells. J Biol Chem. 2005 Feb 11;280(6):4959-67. Epub 2004 Nov 9. PubMed PMID: 15537655.
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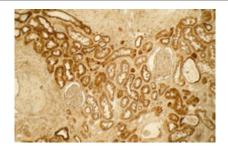


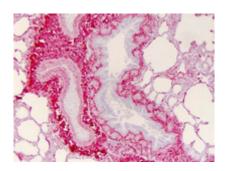
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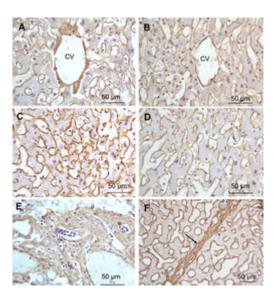
Immunohistochemistry using affinity purified Collagen type I antibody Cat.-No R1038 at a 1/100 dilution to detect distal tubules in normal kidney tissue. Note the absence of staining of glomeruli. The antibody was reacted with antibody for 4 h RT followed by secondary antibody and substrate reaction.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

Immunohistochemistry of human lung tissue (Formalin-fixed, Paraffinembedded) 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.

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 biotinstreptavidin 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.



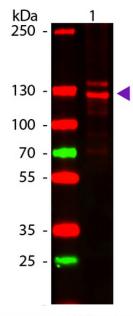






Western Blot of Human Collagen Type I using Collagen I Antibody (Cat.-No R1038).

Lane 1: Human Collagen Type 1. Lane 2: None. Load: 50 ng per lane. Primary antibody: Collagen Type I antibody at 1:1,000 overnight at 4°C. Secondary antibody: DyLight™ 649 rabbit secondary antibody at 1:20,000 for 30 min at RT. Block: MB-070 for 30 min at RT. Predicted/Observed size: 139 & 130 kDa, 139 & 130 kDa for Collagen Type I. Other Band(s): Collagen Type I splice variants and isoforms.



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.

