Datasheet for ABIN5596819

anti-Collagen Type I antibody

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Overview

Quantity:	100 µg
Target:	Collagen Type I (COL1)
Reactivity:	Human, Rat, Mouse, Cow, Mammalian
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This Collagen Type I antibody is un-conjugated
Application:	ELISA, Western Blotting (WB), Immunohistochemistry (IHC), Immunoprecipitation (IP)

Product Details

Immunogen:	Immunogen: Collagen Type I from human and bovine placenta Immunogen Type: Native Protein
Isotype:	IgG
Cross-Reactivity (Details):	Typically negligible 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.
Purification:	COLLAGEN I Antibody 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

Product Details

specificities.

Target Details

Target:	Collagen Type I (COL1)
Alternative Name:	COLLAGEN Type I (COL1 Products)
Background:	Synonyms: Collagen Of Skin Tendon And Bone antibody, Collagen Type 1 antibody, Collagen type I alpha 1 antibody, Collagen type I alpha 2 antibody, OI4 antibody, Osteogenesis Imperfecta Type IV antibody 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. Ideal for investigators involved in Cell Biology, Signal Transduction and Stem Cell research. Gene Name: COL1A1
Gene ID:	1277
UniProt:	P02452

Application Details

Application Notes:	Immunohistochemistry Dilution: 1:50 - 1:200 Application Note: 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. Western Blot Dilution: 1:1,000 - 1:10,000 Immunoprecipitation Dilution: 1:100 ELISA Dilution: 1:5,000 - 1:50,000

Application Details

Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1.0 mg/mL
Buffer:	Buffer: 0.125 M Sodium Borate, 0.075 M Sodium Chloride, 0.005 M EDTA, pH 8.0 Stabilizer: None
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

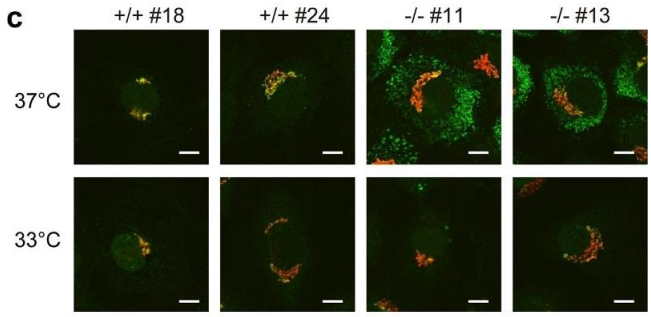
Product cited in:	Keller, Bruch, Schneider, Meier-Hubberten, Hafner, Rudolf: "A Scaffold-Free 3-D Co-Culture Mimics the Major Features of the Reverse Warburg Effect In Vitro." in: **Cells**, Vol. 9, Issue 8, (2020) (PubMed). Thomas, Ahangar, Hofma, Strudwick, Fitridge, Mills, Cowin: "Attenuation of Flightless I Increases Human Pericyte Proliferation, Migration and Angiogenic Functions and Improves Healing in Murine Diabetic Wounds." in: **International journal of molecular sciences**, Vol. 21, Issue 16, (2020) (PubMed). Roth, Enström, Aghabeick, Carlsson, Genové, Paul: "Parenchymal pericytes are not the major contributor of extracellular matrix in the fibrotic scar after stroke in male mice." in: **Journal of neuroscience research**, Vol. 98, Issue 5, pp. 826-842, (2020) (PubMed). Rigon, Hörner, Straka, Bieback, Gretz, Hafner, Rudolf: "Effects of ASC Application on Endplate Regeneration Upon Glycerol-Induced Muscle Damage." in: **Frontiers in molecular neuroscience**

, Vol. 13, pp. 107, (2020) (PubMed).

Fujiwara, Funaki, Fukui, Kimura, Kanou, Ose, Minami, Shintani: "Effects of pirfenidone targeting the tumor microenvironment and tumor-stroma interaction as a novel treatment for non-small cell lung cancer." in: **Scientific reports**, Vol. 10, Issue 1, pp. 10900, (2020) (PubMed).

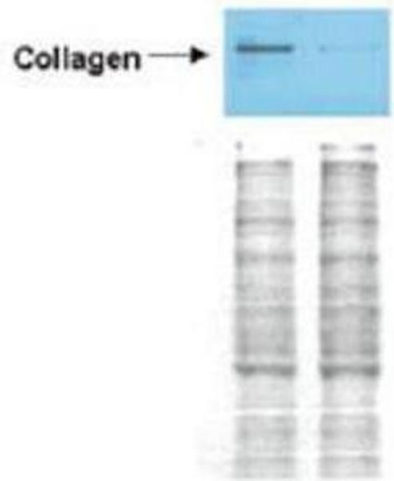
There are more publications referencing this product on: Product page

Validation report #101369 for Immunofluorescence (IF)



Immunofluorescence (Cultured Cells)

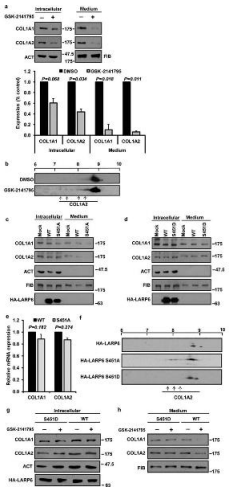
Image 1. Immunofluorescence staining of extracellular and intracellular type I collagen. (a) Immunofluorescence staining of type I collagen secreted from MEF clones was performed with an anti-type I collagen antibody without cell permeabilization. Scale bars: 100 µm. (b,c) Immunofluorescence staining of permeabilized MEF clones was performed with anti-type I collagen (green) and anti-KDEL antibodies (red) (b) or anti-type I collagen (green) and anti-GM130 antibodies (red). (c) Scale bars: 10 µm. - figure provided by CiteAb. Source: PMID31758055



Western Blotting

Image 2. Western blot analysis is shown using Affinity Purified anti-Collagen I antibody to detect expression of collagen I in Wistar rat hepatic stellate cells (HSC) in control (GFP-transduced) (left lane) and PPARg-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 nitro-cellulose membranes. Proteins were detected by incubating the membrane with anti-Collagen 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.



Western Blotting

Image 3. http://www.doi.org/10.1038/srep22597: Reduced secretion of type I collagen by Akt inhibition and S451A overexpression. (a) Top panels: the level of collagen α1(I) (COL1A1) and collagen α2(I) (COL1A2) polypeptides in HLFs was analyzed intracellularly and in the cellular medium by Western blotting after DMSO (−) or Akt inhibiton by GSK-2141795 (+). Loading controls: β-actin (ACT) and fibronectin (FIB). Bottom panel: Western blots from 3 independent experiments as shown in top panels were quantified, normalized to β-actin (for intracellular collagen) and fibronectin (for medium collagen) and expressed as percentage of control cells. Error bars: standard deviation (SD) (n = 3). (b) Hyper-modifications of collagen α2(I) polypeptide after Akt inhibition analyzed by 2DGE and Western blotting. Hyper-modifications are indicated by arrows. The scale on the top indicates pH. (c) Dominant negative effect of S451A mutant on secretion of type I collagen. COL1A1 and COL1A2 polypeptides were measured in cellular extracts (lanes 1–3) and medium (lanes 4–6) of HLFs overexpressing wt HA-LARP6, S451A mutant or in mock transfected cells. Loading controls: β-actin (ACT) and fibronectin (FIB). HA-LARP6: expression of transfected proteins. (d) Same experiment as in (c), except S451D mutant was analyzed. (e) Expression of collagen mRNAs. Total RNA extracted from cells overexpressing wt and S451A LARP6 was analyzed for expression of COL1A1 and COL1A2 mRNAs by real-time PCR and normalized to β-actin mRNA. Error bars: SD (n = 3). (f) Modifications of collagen

α2(I) polypeptide in cells overexpressing wt HA-LARP6, S451A or S451D mutant analyzed by 2DGE and Western blotting. Hyper-modifications are indicated by arrows and pH scale is on the top. (g) GSK-2141795 has no effect on cellular level of collagen polypeptides. HLFs were transfected with wt HA-LARP6 or S451D mutant and treated with DMSO (-) or GSK-2141795 (+) and collagen polypeptides (COL1A1 and COL1A2) were analyzed in cellular extracts by Western blotting. ACT: β-actin loading control. HA-LARP6: expression of transfected proteins. (h) Rescue of collagen secretion by S451D mutant. The medium from cells in (g) was analyzed for collagen polypeptides (COL1A1 and COL1A2) by Western blotting. FIB: fibronectin loading control.

Please check the product details page for more images. Overall 41 images are available for ABIN5596819.

Validation report #103813 for Unfolding Profile (UP)

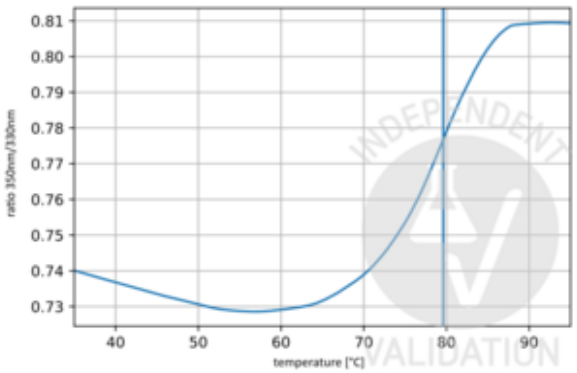
Successfully validated (Unfolding Profile (UP))

by NanoTemper Technologies

Report Number: 103813

Date: Jul 23 2019

Target:	COL1
Lot Number:	41897
Method validated:	Unfolding Profile (UP)
Positive Control:	ABIN5596819
Notes:	Passed. ABIN5596819 showed T_i at 79.6°C and a clear unfolding profile with one unfolding event. This suggests that the antibody is properly folded and functional.
Protocol:	• Dilute ABIN5596819 in PBS buffer (Roth, 1058.1, lot 285231988) to get a final volume of 30µl at a concentration of 0.1µM. • Load sample into Tycho capillary (NanoTemper Technologies, TY-C001). • Run Tycho measurement.
Experimental Notes:	Tycho is designed to run quick and precise protein quality check experiments. Tycho uses intrinsic protein fluorescence to follow protein unfolding while running a fast thermal ramp, yielding results in 3min. A protein's unfolding behavior is characterized by various parameters, most notably the inflection temperature (T_i). The T_i can be used to identify properly folded protein, to compare different batches, or to analyze the influence of storage/transport conditions on a protein. An absence of T_i would suggest that the protein is already unfolded and therefore most likely nonfunctional.



Validation image no. 1 for anti-Collagen, Type I (COL1) antibody (ABIN5596819)

Unfolding profile of ABIN5596819. The fluorescence signal is plotted against temperature. The vertical line indicates the T_i at 79.6 °C.

Validation report #300048 for Immunohistochemistry (IHC)

Successfully validated (Immunohistochemistry (IHC))

by MS Validated Antibodies

Report Number: 300048

Date: Aug 07 2023

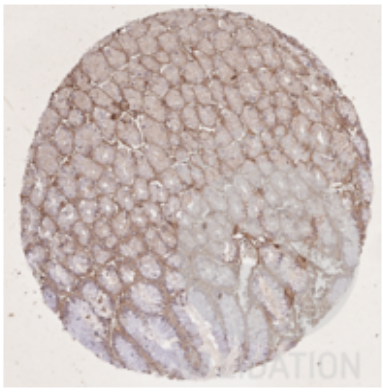
Target:	COL1
Lot Number:	47973
Method validated:	Immunohistochemistry (IHC)
Positive Control:	Human TMA Monoclonal rabbit anti-human COL1A2 monoclonal antibody (HL2048)
Notes:	Passed. Staining of collagen type I using ABIN5596819 is consistent with the expected staining pattern.
Primary Antibody:	ABIN5596819
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 type I antibody (antibodies-online, ABIN5596819, lot 47973) diluted 1:15 or 1:50 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).

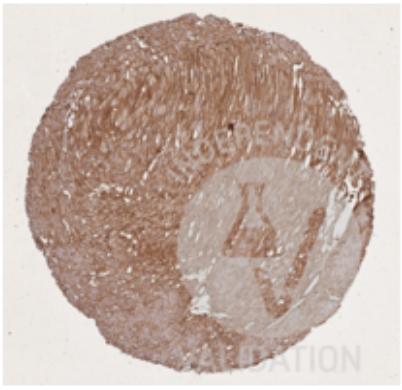
Experimental Notes:

- 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. For ABIN5596819 the staining was typically fibrillar. Positive fibrillar structures were often located adjacent to benign and malignant epithelial structures, in smooth muscles or around vessels of all sizes.
- The anti-collagen type I antibody ABIN5596819 shows a predominantly fibrillar staining pattern involving stroma components that are often located adjacent to benign and malignant epithelial structures, in smooth muscle or around vessels of all sizes. Collagen I staining is also seen in the stroma of many tumors. The staining of these fibrillar structures by ABIN5596819 is often faint at a dilution of 1:50. Staining of fibrils by ABIN5596819 is stronger at a dilution of 1:15 but in this case a significant background staining occurs in many epithelial tissues. Of note, a membranous staining of intratubular testicular cells was seen by ABIN5596819. Considering the expected staining pattern of an anti-collagen type I antibody, this may represent a cross-reactivity of ABIN5596819.
- According to the rather ubiquitous nature of collagen I 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/ENSG00000108821-COL1A1/tissue), collagen I staining by ABIN5596819 was significant in the placenta, smooth muscle, urinary bladder and the endometrium. These are the tissues with the highest recorded RNA expression among the tissues analyzed in this project.



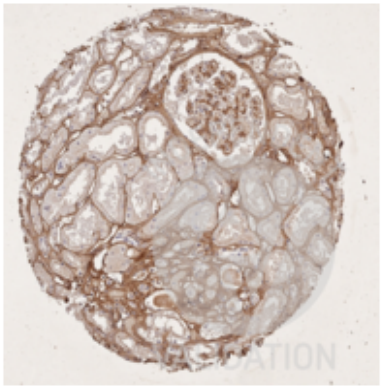
Validation image no. 1 for anti-Collagen, Type I (COL1) antibody (ABIN5596819)

IHC staining of the stomach mucosa with collagen type I antibody ABIN5596819 diluted 1:15 shows staining of basement membranes and the stomach mucosa.



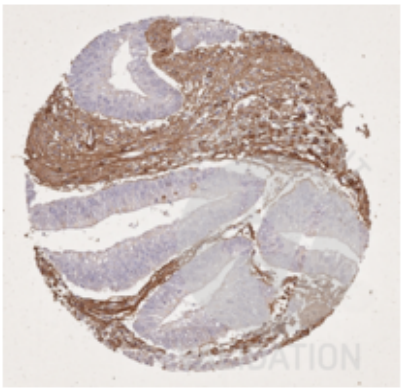
Validation image no. 2 for anti-Collagen, Type I (COL1) antibody (ABIN5596819)

IHC staining of stomach muscular wall with collagen type I antibody ABIN5596819 diluted 1:15 shows collagen I fibres surrounding smooth muscle cells.



Validation image no. 3 for anti-Collagen, Type I (COL1) antibody (ABIN5596819)

IHC staining of kidney with collagen type I antibody ABIN5596819 diluted 1:15 shows intense staining of fibres surrounding tubuli and around blood vessels.



Validation image no. 4 for anti-Collagen, Type I (COL1) antibody (ABIN5596819)

IHC staining of colorectal carcinoma with collagen type I antibody ABIN5596819 diluted 1:15 shows dense fibrillar collagen I deposits in the stroma. Cancer cells are collagen I negative.