

**Datasheet for 600-401-108-0.1****Collagen Type VI Antibody****Overview**

<b>Description:</b>	Anti-Collagen Type VI (RABBIT) Antibody - 600-401-108-0.1
<b>Item No.:</b>	600-401-108-0.1
<b>Size:</b>	100 µg
<b>Applications:</b>	Dot Blot, IHC, IF, Multiplex, WB
<b>Reactivity:</b>	Human, Bovine
<b>Host Species:</b>	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.

<b>Synonyms:</b>	rabbit anti-Collagen Type VI antibody, Collagen alpha-1 (VI) chain, Collagen VI antibody, Human mRNA for collagen VI alpha 1 C terminal globular domain antibody
<b>Host Species:</b>	Rabbit
<b>Clonality:</b>	Polyclonal
<b>Format:</b>	IgG

**Target Details**

<b>Gene Name:</b>	COL6A1-COL6A3
<b>Reactivity:</b>	Human, Bovine

<b>Immunogen Type:</b>	Native Protein
<b>Immunogen:</b>	Collagen Type VI from human and bovine placenta
<b>Purity/Specificity:</b>	Anti-Collagen Type VI 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. 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 VI collagens and has negligible cross-reactivity with Type I, II, III, IV or V collagens. Non-specific cross-reaction of anti-collagen antibodies with other human serum proteins or non-collagen extracellular matrix proteins is negligible.
<b>Relevant Links:</b>	<ul style="list-style-type: none"><li>• <a href="#">Anti-Collagen IHC Protocol</a></li><li>• <a href="#">UniProtKB - P12111</a></li><li>• <a href="#">UniProtKB - P12110</a></li><li>• <a href="#">NCBI - AAH52575.1</a></li><li>• <a href="#">UniProtKB - P12109</a></li><li>• <a href="#">GeneID - 1291</a></li></ul>

## Application Details

<b>Tested Applications:</b>	Dot Blot, IHC
<b>Suggested Applications:</b>	IF, Multiplex, WB (Based on references)
<b>Application Note:</b>	Anti-Collagen Type VI has been tested by dot blot and IHC-P 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.
<b>Assay Dilutions:</b>	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
<b>ELISA:</b>	1:5,000 - 1:20,000
<b>IHC:</b>	1:50 - 1:200
<b>IP:</b>	1:100
<b>WB:</b>	1:500 - 1:5,000

## Formulation

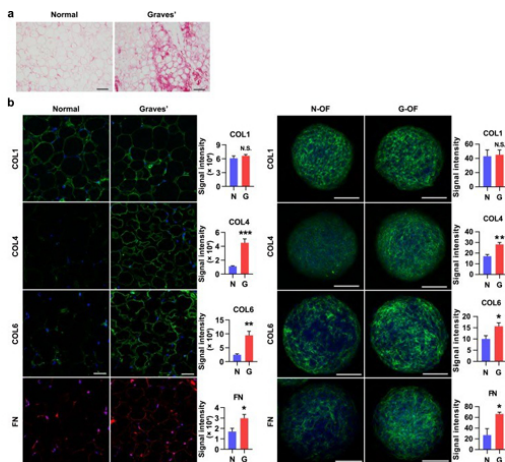
<b>Physical State:</b>	Liquid (sterile filtered)
------------------------	---------------------------

<b>Concentration:</b>	1.19 mg/mL by UV absorbance at 280 nm
<b>Buffer:</b>	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
<b>Preservative:</b>	0.01% (w/v) Sodium Azide
<b>Stabilizer:</b>	None

## Shipping & Handling

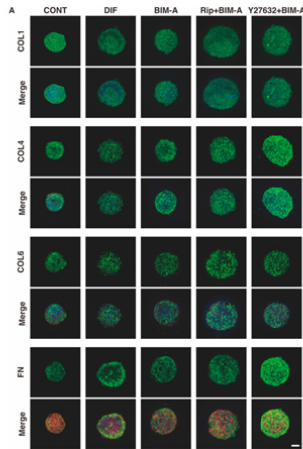
<b>Shipping Condition:</b>	Wet Ice
<b>Storage Condition:</b>	Store vial at 4° C prior to opening. This product is stable at 4° C as an undiluted liquid. Dilute only prior to immediate use. For extended storage, mix with an equal volume of glycerol, aliquot contents and freeze at -20° C or below. Avoid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

## Images



### Immunofluorescence Microscopy

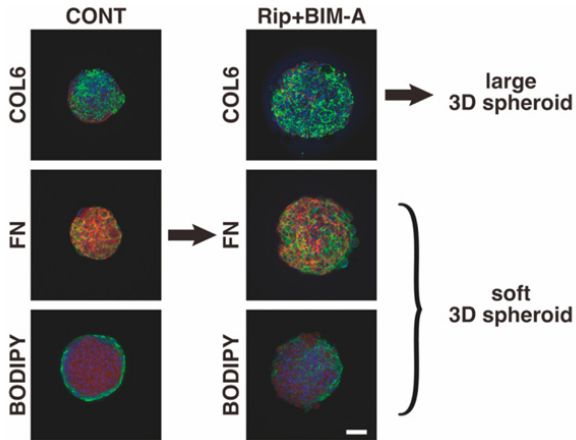
Unique ECM deposition in GO tissues and GO-derived OF organoids. (a) Representative Sirius red staining of periocular adipose tissues in control (normal) and GO (Graves). Scale bars, 100  $\mu$ m. (b) Representative immunofluorescent staining of collagens (green) and FN (red or green) with DAPI (blue) in control and Graves orbital adipose tissues (left, n = 4 to 5) and 3D organoids reconstituted from hOFs isolated from respective tissues (right). Signal intensities were quantified per spheroid. n = 5 to 8 organoids. Scale bars, 50  $\mu$ m (left) and 100  $\mu$ m (right). G indicates G-OF; N indicates N-OF. \*P < 0.05, unpaired Student t test (n = 4 in each group). Fig 2. PMID: 30388216



**Immunofluorescence Microscopy**

Representative confocal images showing the expression of ECMs in 3D 3T3-L1 spheroids under several sets of conditions. (A) On Day 7, the 3D cultures of spheroids of 3T3-L1 preadipocytes as the control (CONT), and their adipogenic differentiation in the absence (DIF) or presence of 100 nM bimatoprost free acid (BIM-A) and/or 10 μM ROCK-i (Rip or Y27632), were immunostained with specific antibodies of ECMs designated by the green color. Scale bar: 100 μm. Fig 9.

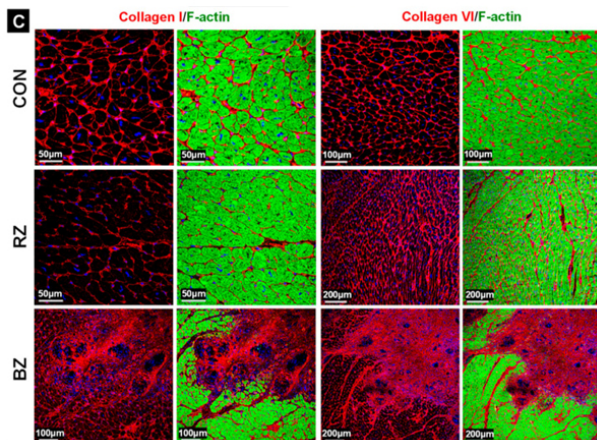
PMID: 36421103



**Immunofluorescence Microscopy**

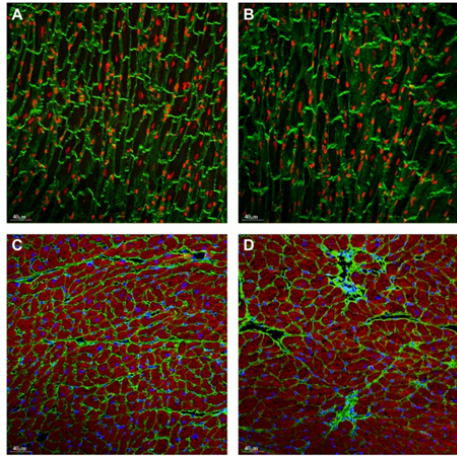
Hypothetical model of the mechanism responsible for inducing huge-sized and soft 3D spheroids caused by the addition of Rip to BIM-A. Fig 10.

PMID: 36421103



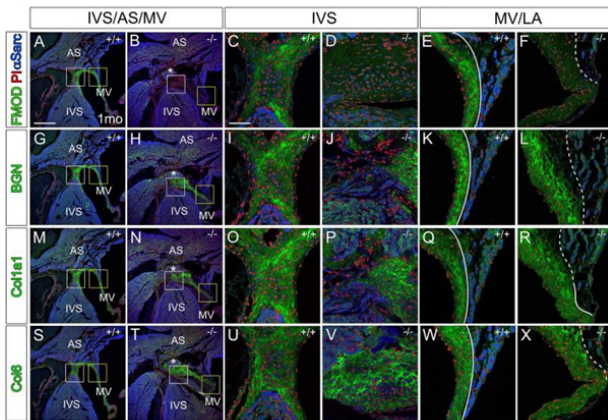
**Immunofluorescence Microscopy**

(C) Staining of collagen I and VI mark fibrotic areas. For comparison, the control (CON) tissue is shown. Border zone (BZ) is the myocardial area surrounding the granuloma and remote zone (RZ) is the myocardium distant to granuloma. The nuclei are stained blue with DAPI. Fig 1. PMID: 33923774



### Immunofluorescence Microscopy

Representative vinculin staining (green) in WT (PBS) mice (A) and WT doxorubicin (B). Nuclei (red) were visualized with drq5. Replacement fibrosis was detected in the doxorubicin treated hearts (D) but not in vehicle hearts (C), visualising with the anti-collagen VI antibody (green). Nuclei (blue) were visualised with DAPI, (E, F). Fig 2. PMID: 28662206



### Immunofluorescence Microscopy

Immunolocalization of FMOD (green, A-F), BGN (green, G-L), Col1a1 (green, M-R), and Col6 (green, S-X) in *Fmod*<sup>+/+</sup> (A, C, E, G, I, K, M, O, Q, S, U, W) and *Fmod*<sup>-/-</sup> (B, D, F, H, J, L, N, P, R, T, V, X) mitral leaflet attachments. White boxes in A, B, G, H, M, N, S, T are magnified in panels C, D, I, J, O, P, U, V respectively. Yellow boxes in A, B, G, H, M, N, S, T are magnified and shown in E, F, K, L, Q, R, W, X respectively. Red- propidium iodide (PI); blue-  $\alpha$ -sarcomeric actin ( $\alpha$ Sarc); IVS-interventricular septum; AS- atrial septum; MV- mitral valve; LA- left atrium; solid lines- distinct separation of MV and LA; dashed lines- diffuse attachment of left atrial to MV. Scale bars: A= 400 $\mu$ m applies to B, G, H, M, N, S, T; C= 50 $\mu$ m applies to D-F, I-L, O-R, U-X. Fig 3. PMID: 27503167

### Dot Blot

Dot Blot results using Rabbit Anti-Collagen VI Antibody.

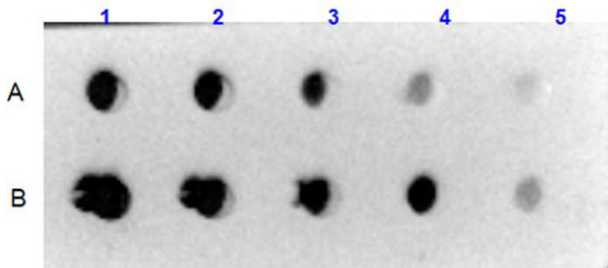
Sample: A) Human Collagen VI (p/n 009-001-108), B) Bovine/Human Collagen VI (p/n 001-001-108).

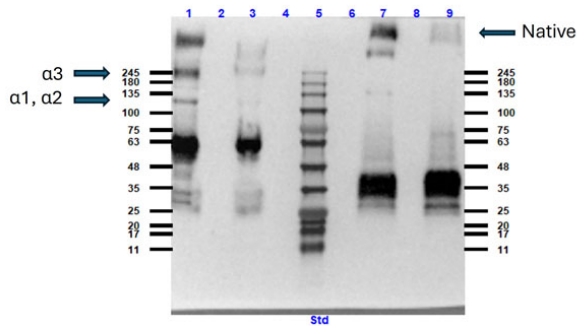
Load: 1) 1000ng, 2) 333.33ng, 3) 111.11ng, 4) 37.03ng, 5) 12.35ng.

Primary Antibody: Rabbit Anti-Collagen VI antibody (p/n 600-401-108) at 5 $\mu$ g/mL at RT for 1hr.

Secondary Antibody: Goat Anti-Rabbit IgG HRP Conjugated (p/n 611-1302) 1:40,000 at RT for 30mins.

Blocking: BlockOut<sup>®</sup> Universal Blocking Buffer (p/n MB-073) at RT for 30mins.





### Western Blot

Western blot results using Rabbit Anti-Collagen Type VI Antibody.

Lane: 1) Human Collagen Type VI (p/n 009-001-108) reduced and boiled, 3) Human Collagen Type VI reduced and not boiled, 5) Opal prestain molecular weight marker (p/n MB-210-0500), 7) Human Collagen Type VI not reduced and boiled, 9) Human Collagen Type VI not reduced and not boiled.

Lanes 2, 4, 6, 8 blank.

Load: 10µg.

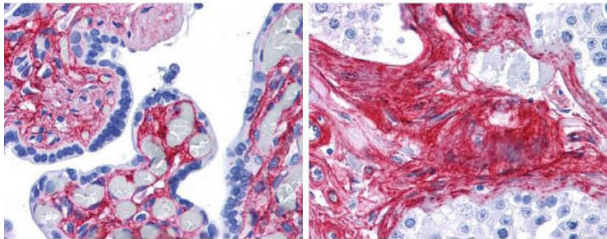
Primary Antibody: Rabbit Anti-Collagen Type VI Antibody (p/n 600-401-108) 1:500 overnight at 2-8°C.

Secondary Antibody: Goat anti-Rabbit IgG HRP conjugated (p/n 611-1302) 1:40,000 at RT for 30mins.

Blocking: BlockOut® Universal Blocking Buffer (p/n MB-073) at RT for 1hr.

### Immunohistochemistry

Rockland anti collagen VI antibody (600-401-108 Lot 26009, 1:400 45 min RT) showed strong staining in FFPE sections of human placenta (Left) with red staining of stromal and extracellular spaces, and in testis (Right) with staining of extracellular spaces between seminiferous tubules). 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



## References

- Caputo A et al. Spatial Transcriptomics Suggests That Alterations Occur in the Preneoplastic Breast Microenvironment of BRCA1/2 Mutation Carriers. *Mol Cancer Res.* (2024)
- Ida Y et al. Addition of ROCK Inhibitors Alleviates Prostaglandin-Induced Inhibition of Adipogenesis in 3T3L-1 Spheroids. *Bioengineering (Basel).* (2022)
- Ida, Y et al. ROCK 1 and 2 affect the spatial architecture of 3D spheroids derived from human corneal stromal fibroblasts in different manners. *Scientific Reports* (2022)
- Suzuki, S et al. Comparison of the Drug-Induced Efficacies between Omidenepag Isopropyl, an EP2 Agonist and PGF2 $\alpha$  toward TGF- $\beta$ 2-Modulated Human Trabecular Meshwork (HTM) Cells. *Journal of Clinical Medicine* (2022)
- Gajawada P et al. Myocardial Accumulations of Reg3A, Reg3 $\gamma$  and Oncostatin M are Associated With the Formation of Granulomata in Patients With Cardiac Sarcoidosis. *Int J Mol Sci.* (2021)
- Ida Y. et al. Prostaglandin F2 $\alpha$  agonist-induced suppression of 3T3-L1 cell adipogenesis affects spatial formation of extracellular matrix. *Sci Rep* (2020)
- Hikage F, Atkins S, Kahana A, Smith TJ, Chun TH. HIF2A-LOX Pathway Promotes Fibrotic Tissue Remodeling in Thyroid-Associated Orbitopathy. *Endocrinology.* (2019)
- Akama T, Chun TH. Transcription factor 21 (TCF21) promotes proinflammatory interleukin 6 expression and extracellular matrix remodeling in visceral adipose stem cells. *J Biol Chem.* (2018)
- Piotrowska I, Isalan M, Mielcarek M. Early transcriptional alteration of histone deacetylases in a murine model of doxorubicin-induced cardiomyopathy. *PLoS One.* (2017)
- Hayes AJ, Shu CC, Lord MS, Little CB, Whitelock JM, Melrose J. Pericellular colocalisation and interactive properties of type VI collagen and perlecan in the intervertebral disc. *Eur Cell Mater.* (2016)
- Dupuis LE, Doucette L, Rice AK, et al. Development of myotendinous-like junctions that anchor cardiac valves requires fibromodulin and lumican. *Dev Dyn.* (2016)
- Chun TH, Inoue M. 3-D adipocyte differentiation and peri-adipocyte collagen turnover. *Methods Enzymol.* (2014)
- Cao L, Guilak F, Setton LA. Three-dimensional morphology of the pericellular matrix of intervertebral disc cells in the rat. *J Anat.* (2007)

## Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.