ROCKLAND
Immunochemicals for Research
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Certificate of Analysis

Product: Affinity Purified Anti-Collagen Type I [Rabbit] Minimum Cross Reactivity to Type II, III, IV, V and VI Collagens

Code: 600-401-103-0.5 **Lot** # 20289

Size: 500 µg

Physical State: Liquid (sterile filtered)

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

Buffer: 0.125M Sodium Borate, 0.075M Sodium Chloride, 0.005M EDTA; pH 8.0

Stabilizer: None **Preservative:** 0.01% (w/v) Sodium Azide

Background(s): 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.

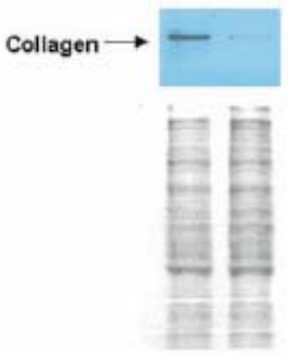

Figure. Western blot analysis is shown using Rockland's Affinity Purified anti-Collagen I antibody to detect expression of collagen I in Wistar rat hepatic stellate cells (HSC) in control (GFP-transduced) (left lane) and PPARγ-transduced cell lysates (right lane). Protein staining shown *below* each 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 (0.1 M 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.*

Application(s): 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. For immunohistochemistry, prepare tissues as frozen tissue sections.

Recommended Dilution(s):

ELISA	1:5,000 - 1:50,000
WESTERN BLOT	1:5,000 - 1:50,000
IMMUNOHISTOCHEMISTRY	1:50 - 1:200
OTHER APPLICATIONS	User Optimized

Storage Conditions: 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. Expiration date is one (1) year from date of opening product.

Purity and 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.

Immunogen: Collagen Type I from human and bovine placenta

Specific Refererence(s):

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.

Hashimoto, N *et al.* (2004) Bone marrow–derived progenitor cells in pulmonary fibrosis. *J. Clin. Invest.* **113**:243-252.

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.

She, H, Xiong, S, Hazra, S, Tsukamoto, H (2005) Adipogenic transcriptional regulation of hepatic stellate cells. *J. Biol. Chem.* **280**(6):4959-4967.

Related Products:

#600-401-103-0.1	Affinity Purified Anti-Collagen Type I (RABBIT)	
#600-401-104-0.1	Affinity Purified Anti-Collagen Type II (RABBIT)	
#600-401-105-0.1	Affinity Purified Anti-Collagen Type III (RABBIT)	
#600-401-106-0.1	Affinity Purified Anti-Collagen Type IV (RABBIT)	
#600-401-107-0.1	Affinity Purified Anti-Collagen Type V (RABBIT)	
#600-401-108-0.1	Affinity Purified Anti-Collagen Type VI (RABBIT)	
#611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10	
#611-132-122	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10	
#B501-0500	500 g	BLOTTO
#BSA-30	500 ml	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer)
#B304	10 ml	NORMAL GOAT SERUM (NGS)
#MB-070	Blocking Buffer for Fluorescent Western Blotting	
#KIA-003	***Max*Tag**™ Anti-RABBIT IgG Kit for Immunoblotting	

Note: 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.

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