ROCKLAND
Immunochemicals for Research

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Certificate of Analysis

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

Code: 600-401-104-0.5 **Lot** # 18852

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 of SDS-PAGE and immunoblotting are used.

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.

Recommended Dilution(s): This product was assayed by immunoblot and found to be reactive against Collagen II at a dilution of 1:5,000 to 1:10,000. This product was also assayed against 1.0 μg of Collagen II in a standard sandwich ELISA using Peroxidase conjugated Affinity Purified anti-Rabbit IgG [H&L] (Goat) (code #611-1302) and (ABTS (2,2'-azino-bis-[3-ethylbenthiazoline-6-sulfonic acid]) (code # ABTS-100) as a substrate for 30 minutes at room temperature. A working dilution of 1:4,000 to 1:8,000 of the stock concentration is suggested for this product. For immunohistochemistry on frozen tissue sections dilute the product 1:50 to 1:200. Optimal titers for other applications should be determined by the researcher.

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 II collagens and has negligible cross-reactivity with Type I, 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 II from human knee cartilage and bovine nasal cartilage.

USDA Certification: 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.

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.

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