

Collagen IV

Concentrated and Prediluted Monoclonal Antibody
901-112-110917

BIOCARE
M E D I C A L

Catalog Number:	CM 112 B	PM 112 AA
Description:	0.5 ml, concentrated	6.0 ml, prediluted
Dilution:	1:100	Ready-to-use
Diluent:	Van Gogh Yellow	N/A

Intended Use:

For In Vitro Diagnostic Use

Collagen IV [Col 94] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of type IV collagen by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

This antibody reacts with type IV collagen, which is the major constituent of the basement membranes. Collagen IV antibody stains the basement membranes in a variety of tissues including kidney, muscle, lymph nodes, lung, tendon and spleen. Collagen IV has been shown to be useful in differentiating microinvasive from *in situ* ductal carcinomas of the breast. Other collagen IV studies include pancreatic adenocarcinoma and chronic pancreatitis, nephrosclerosis and other kidney diseases, oral squamous cell carcinoma, laryngeal cancers and ovarian cancers.

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: Col 94

Isotype: IgG1

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.

Epitope/Antigen: Type IV collagen

Cellular Localization: Basement membrane

Positive Tissue Control: Skin or kidney

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations:

Peroxide Block: Block for 5 minutes with Biocare's Peroxidized 1.

Digestion Method:

Digest with Pepsin enzyme for 5 minutes at 37°C -or- for 15 minutes at RT.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: Incubate for 10 minutes at RT with a secondary probe.

Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

Protocol Recommendations:

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB - OR - Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:

This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (7)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

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References:

1. Nakano S, *et al.* Differential tissular expression and localization of type IV collagen alpha1(IV), alpha2(IV), alpha5(IV), and alpha6(IV) chains and their mRNA in normal breast and in benign and malignant breast tumors. *Lab Invest.* 1999 Mar;79(3):281-92.
2. Damiani S, *et al.* Myoepithelial cells and basal lamina in poorly differentiated in situ duct carcinoma of the breast. An immunocytochemical study. *Virchows Arch.* 1999 Mar;434(3):227-34.
3. Firth NA, Reade PC. The prognosis of oral mucosal squamous cell carcinomas: a comparison of clinical and histopathological grading and of laminin and type IV collagen staining. *Aust Dent J.* 1996 Apr;41(2):83-6.
4. Razzaque MS, *et al.* Glomerular expression of type III and type IV collagens in benign nephrosclerosis: immunohistochemical and in situ hybridization study. *Pathol Res Pract.* 1994 May;190(5):493-9. Published erratum appears in *Pathol Res Pract.* 1995 Mar;191(2):179.
5. Lee CS, *et al.* Distribution of type IV collagen in pancreatic adenocarcinoma and chronic pancreatitis. *Int J Exp Pathol.* 1994 Apr;75(2):79-83.
6. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
7. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.