Monoclonal Mouse Anti-Human Collagen IV
Clone CIV 22
Code No. M 0785
Lot 020. Edition 04.09.02

Intended use
For in vitro diagnostic use.
DAKO Monoclonal Mouse Anti-Human Collagen IV, Clone CIV 22, is intended for use in immunocytochemistry. The antibody labels type IV collagen and is a useful tool for the identification of basement membranes. Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Introduction
Collagen IV is a major constituent in particular of the lamina densa of basement membranes and ultrastructurally it looks amorphous. Basement membranes are thin extracellular matrices separating parenchymal, endothelial, and epithelial cells from underlying connective tissue. In glomerular and tubular basement membranes 40% of the protein is type IV collagen.
Detection of intracellular type IV collagen is generally difficult owing to a low concentration. In newly formed capillaries found in the inflammatory sites of rheumatoid arthritis synovium, intracellular type IV collagen may, however, be detected (1).

Structurally the protein consists of four domains. One of these, the triple helical collagen IV domain, is formed by the association of two polypeptide chains of type α1 and one of type α2. This domain is 340 nm long and is highly crosslinked by disulphide bridges (1, 2).
Diagnostic applications of collagen type IV immunostaining have mostly centered around the demonstration of basal lamina in invasive tumours. In particular, the demonstration of an intact basal lamina has been used to distinguish benign glandular proliferations, such as microglandular adenosis and sclerosing adenosis, from well-differentiated carcinoma, like tubular carcinoma of the breast (3).

Reagent provided
Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 50 mmol/L Tris/HCl, pH 7.2, and containing 15 mmol/L NaNO3. Package size is 1 mL.
Clone: CIV 22 (2). Isotype: IgG1, kappa.
IgG concentration: 75 mg/L. Total protein concentration: 3.3 g/L.

Immunogen
Purified pepsin fragments of type IV collagen isolated from human kidney (2).

Specificity
The specificity of the antibody has been assessed in radioimmunoassay (RIA) where it recognizes an epitope present in the human type IV collagen in native conformation, whereas it does not recognize the reduced and alkylated protein in the denatured state. Staining of immunoblots with the antibody was negative, further indicating that the antibody recognizes a conformational epitope on type IV collagen (2).
No cross reactivity of the antibody with isolated human collagen types I, II, III and V could be detected by RIA or by immunoblotting (2).
As demonstrated by RIA the antibody cross reacts with native bovine type IV collagen (2).

Precautions
1. For in vitro diagnostic use.
2. This product contains sodium azide (Na3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

Storage
Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact DAKO Technical Services.

Specimen preparation
Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin (4). Pre-treatment of tissues with heat-induced epitope retrieval is required. For heat-induced epitope retrieval, the following solutions were found efficient: 10 mmol/L citrate buffer, pH 6.0; 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0; and particularly DAKO Target Retrieval Solution, code No. S 1700. Pre-treatment of tissues with proteinase K was found to be less efficient. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.
Frozen sections and cell preparations: The antibody can be used on frozen sections (5, 6).

**Staining procedure**

**Dilution:** DAKO Monoclonal Mouse Anti-Human Collagen IV, code No. M 0785, may be used at a dilution range of 1:25-1:50 when applied on formalin-fixed, paraffin-embedded sections of kidney and using 20 minutes heat-induced epitope retrieval in DAKO Target Retrieval Solution, code No. S 1700, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is DAKO Mouse IgG1, code No. X 0931, diluted to the same mouse IgG concentration as the primary antibody.

**Visualization:** DAKO LSAB®+/HRP kit, code No. K 0679, and DAKO EnVision™+/HRP kits, code Nos. K 4004 and K 4006, are recommended. Follow the procedure enclosed with the selected visualization kit.

**Automation:** The antibody is well suited for immunocytochemical staining using automated platforms, such as the DAKO Autostainer.

**Performance characteristics**

**Normal tissues:** The antibody shows the characteristic labelling of basement membranes in a variety of tissues and organs tested, including kidney, skin, striated and smooth muscle, spleen, lymph node, lung, placenta and tendon. In spleen and lymph nodes, the expected fragmented labelling of the discontinuous basement membranes of the sinusoids is observed, whereas other blood vessels exhibit a linear, continuous labelling. In kidneys, basement membranes of capillaries, parts of the mesangial matrix and the Bowman’s capsule, and the tubular basement membranes are labelled by the antibody. The only basement membrane showing negative labelling with the antibody is that of the corneal epithelium. All structures others than basement membranes are consistently negative with this antibody (2).

**Abnormal tissues:** In congenital epidermolysis bullosa (EB), the antibody allowed a rapid distinction between the two major variants, EB simplex, and EB dystrophic (4). Around dilated vessels of port-wine stains, the antibody labelled much broader belts than around vessels in normal skin (5), and in angioimmunoblastic lymphadenopathy, vascular proliferation and small amounts of intercellular collagenous fibrils were reliably revealed by the antibody (5).

**References**


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