Novocastra™ Lyophilized Mouse Monoclonal Antibody Collagen Type IV

Product Code: NCL-COLL-IV

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human collagen type IV.
Clone	PHM-12
Ig Class	IgG1
Antigen Used for Immunizations	Human glomeruli.
Hybridoma Partner	Mouse myeloma (p3-NS1-Ag4-1).
Preparation	Lyophilized tissue culture supernatant containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
Effective on Frozen Tissue	Yes
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique followed immediately by trypsin digestion for 30 seconds ONLY, and not 30 minutes as indicated in step 5 of the trypsin protocol: see attached for both techniques).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100–1:200. High temperature antigen unmasking combined with 30 seconds of trypsin digestion. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not evaluated.
Positive Controls	Immunohistochemistry: Kidney; basement membranes. Western Blotting: Not evaluated.
Staining Pattern	Basement membranes.
Storage and Stability	Store unopened lyophilized antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.
General Overview	NCL-COLL-IV recognises collagen IV, which is a major constituent of basement membranes. In kidney, NCL-COLL-IV reacts with glomerular and tubular basement membranes and also mesangial cells and matrix within the glomerulus. The antibody reacts with the basal lamina of capillaries as well as basement membrane structures in all organs examined.
General References	Gutterson B A, et al.. Laboratory Investigation. 51: 82–87 (1984). Hancock W W, et al.. Pathology. 16: 197–206 (1984).

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Instructions for Use

High Temperature Antigen Unmasking Technique Followed by Trypsin Digestion for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf - see other Epitope Retrieval Solutions in the range) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Place slides in pre-heated distilled water to bring the sections to 37 °C for a minimum of 5 minutes.
9. Incubate sections in pre-heated Trypsin solution at 37 °C for 30 seconds.
10. Rinse sections in running tap water.
11. Proceed with immunohistochemistry protocol.
12. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
13. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
14. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate sections in an appropriate biotinylated secondary antibody.
17. Wash in TBS buffer for 2 x 5 minutes.
18. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
19. Wash in TBS buffer for 2 x 5 minutes.
20. Incubate slides in DAB or other suitable peroxidase substrate.
21. Wash thoroughly in running tap water.
22. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

Trypsin Solution

**Trypsin containing chymotrypsin should always be used. The enzyme activities can vary from a supplier and between batches. Such variations may affect the incubation time required.*

Preheat the following to 37 °C using a water bath:

(i) 200 mL of TBS

(ii) 200 mL of distilled water.

Dissolve 0.2 g Trypsin 250 and 0.2 g Calcium Chloride in the 200 mL of TBS.

Once the Trypsin solution is at 37 °C, pH to 7.8 with 1 M sodium hydroxide.

0.01 M Citrate Buffer (pH 6.0) or RE7113 (where available).

Add 3.84 grams of Citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available)

Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 L of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/0.65 mM EDTA/0.0005% TWEEN (pH 9.0) or RE7119 (where available)

Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

* *In most applications, 10mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ THE MANUFACTURER'S INSTRUCTIONS.