

Novocastra™ Liquid Mouse Monoclonal Antibody Cytokeratin 20

Product Code: NCL-L-CK20-561

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human cytokeratin 20 intermediate filament protein.
Clone	PW31
Ig Class	IgG1
Antigen Used for Immunizations	A prokaryotic recombinant protein containing a 70 amino acid region from the N-terminal of the cytokeratin 20 intermediate filament protein.
Hybridoma Partner	Mouse myeloma (p3-NS1-Ag4-1).
Preparation	Liquid tissue culture supernatant containing 15 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Not evaluated.
Effective on Paraffin Wax Embedded Tissue	Yes (using heat induced epitope retrieval with Citrate-based buffer, pH 6.0: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100. Citrate-based buffer, pH 6.0. 30 minutes primary antibody incubation at 25 °C. Polymer detection recommended. Western Blotting: Typical working dilution 1:5. (ECL™, Amersham Pharmacia Biotech).
Positive Controls	Immunohistochemistry: Colon. Western Blotting: Ht29-219, CaCo ₂ .
Staining Pattern	Cytoplasmic.
Storage and Stability	Store liquid 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. Prepare working dilutions on the day of use.
General Overview	Cytokeratin 20 has been demonstrated to be almost entirely confined to the gastric and intestinal epithelium, urothelium and Merkel cells of the skin. Cytokeratin 20 is less acidic than other type I cytokeratins and is of interest due to its restricted tissue expression. In normal tissue, cytokeratin 20 is reported to be expressed in intestinal epithelium, gastric foveolar epithelium, a number of endocrine cells in the upper portions of the pyloric glands, urothelium and Merkel cells in epidermis.
General References	Campbell F and Herrington C S. Current Diagnostic Pathology. 7 113–122 (2001). Leech S N, Kolar A J O, Barrett P D et al.. Journal of Clinical Pathology. 54 727–729 (2001).



Instructions for Use

Heat Induced Epitope Retrieval Combined With Polymer Detection 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 epitope retrieval solution (Citrate based pH 6.0 - Epitope Retrieval Solution unless otherwise indicated overleaf) in a stainless steel pressure cooker until boiling. 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 epitope retrieval 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. Wash sections once using fresh Tris-Buffered Saline (TBS, pH 7.6) buffer for 5 minutes.
9. Place sections in diluted normal serum (eg NCL-G-SERUM) for 10 minutes.
10. Incubate sections with primary antibody.
11. Wash twice, each time using fresh TBS buffer for 5 minutes.
12. For visualization of the bound primary antibody, follow instructions supplied with the Polymer Detection System.
13. Counterstain with hematoxylin (if required), dehydrate and mount.

* (In most applications, Phosphate Buffered Saline, pH 7.6, can be used instead of TBS, pH 7.6).

Safety Note

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