Novocastra™ Liquid Mouse Monoclonal Antibody Cytokeratin 5

Product Code: NCL-L-CK5

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human cytokeratin 5 intermediate filament protein
Clone	XM26
Ig Class	IgG1, kappa
Antigen Used for Immunizations	Prokaryotic recombinant fusion protein corresponding to a 103 amino acid portion of the C-terminal region of the human cytokeratin 5 molecule.
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	Yes. Acetone fixation recommended.
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: 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 1:500–1:1000.
Positive Controls	Immunohistochemistry: Prostate. Western Blotting: A431 cell line.
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	Cytokeratins are a large family of cytoskeletal proteins found in epithelial cells. They are co-ordinately synthesized in pairs so that at least one member of each family is expressed in each epithelial cell. Cytokeratins assemble into obligatory heteropolymers composed of type I (acidic) and type II (basic) polypeptides to form higher order tetramers and protofilaments. Basal cells of human epidermis express acidic keratin 14 and basic cytokeratin 5. Cytokeratin 5 is a 58 kD protein that is closely related to cytokeratin 6. They share similar tissue distribution and are reported to be found in various proportions in many non-keratinizing stratified squamous epithelia eg tongue mucosa, as well as in basal epithelia of trachea, basal cells of epidermis, hair follicles, sebaceous and sweat glands of skin, luminal cells of the mammary gland, basal cells of prostate, urothelium, vagina and endocervical mucosa.
General References	Whittock N V, Eady R A and McGrath J A. Biochem. Biophys. Res. Commun. 274 (1): 149–152 (2000). Irvine A D, McKenna K E, Jenkinson H, et al.. Journal of Investigative Dermatology. 108 (5): 809–810 (1997). Morley S M, Dundas S R, James J L, et al.. Journal of Cell Science. 108 (Pt 11): 3463–3471 (1995). Stephens K, Zlotoforski A, Smith L, et al.. American Journal of Human Genetics. 56 (3): 577–585 (1995). Chan Y M, Yu Q C, LeBlanc-Straceski J, et al.. Journal of Cell Science. 107 (Pt 4): 765–774 (1994). Coulombe P A, Chan Y M, Albers K, et al.. Journal of Cell Biology. 111 (6 Pt 2): 3049–3064 (1990). Moll R, Dhouailly D and Sun T-T. Virchows Archiv B Cell Pathol. 58: 129–145 (1989). Moll R, Franke W W and Schiller D L. Cell. 31: 11–24 (1982).

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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.