

# Novocastra™ Ready-to-Use Mouse Monoclonal Antibody Cytokeratin 5

**Leica**  
BIOSYSTEMS

## Product Code: RTU-CK5

<b>Intended Use</b>	FOR RESEARCH USE ONLY.
<b>Specificity</b>	Human cytokeratin 5 intermediate filament protein.
<b>Clone</b>	XM26
<b>Ig Class</b>	IgG1, kappa
<b>Antigen Used for Immunizations</b>	Prokaryotic recombinant fusion protein corresponding to a 103 amino acid portion of the C-terminal region of the human cytokeratin 5 molecule.
<b>Hybridoma Partner</b>	Mouse myeloma (p3-NS1-Ag4-1).
<b>Preparation</b>	Tissue culture supernatant diluted in 5% horse serum in PBS containing 12 mM sodium azide. Volume as indicated on vial label.
<b>Effective on Frozen Tissue</b>	Yes. Acetone fixation recommended.
<b>Effective on Paraffin Wax Embedded Tissue</b>	Yes (using the high temperature antigen unmasking technique: see overleaf).
<b>Recommendations on Use</b>	Immunohistochemistry: Typical working dilution: NEAT. 15 minutes primary antibody incubation at 25 °C when used in conjunction with the Novostain Universal Detection Kit (Ready to Use), code NCL-RTU-D. Recommendations on use will differ if other detection systems are used eg Standard ABC technique. Western Blotting: Not recommended. Not recommended for use on Ventana automated staining systems (Ventana Medical Systems inc., USA).
<b>Positive Controls</b>	Immunohistochemistry: Prostate.
<b>Staining Pattern</b>	Cytoplasmic.
<b>Storage and Stability</b>	Store ready-to-use prediluted liquid antibody at 4 °C. Return to 4 °C immediately after use. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label.

<b>General Overview</b>	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.
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<b>General References</b>	Whitlock NV, Eady RA and McGrath JA. <i>Biochem. Biophys. Res. Commun.</i> 274 (1): 149–152 (2000). Irvine A D, McKenna K E, Jenkinson H, et al.. <i>Journal of Investigative Dermatology.</i> 108 (5): 809–810 (1997). Morley S M, Dundas S R, James J L, et al.. <i>Journal of Cell Science.</i> 108 (Pt 11): 3463–3471 (1995). Stephens K, Zlotoforski A, Smith L, et al.. <i>American Journal of Human Genetics.</i> 56 (3): 577–585 (1995). Chan Y M, Yu Q C, LeBlanc-Straceski J, et al.. <i>Journal of Cell Science.</i> 107 (Pt 4): 765–774 (1994). Coulombe P A, Chan Y M, Albers K, et al.. <i>Journal of Cell Biology.</i> 111 (6 Pt 2): 3049–3064 (1990). Moll R, Dhouailly D and Sun T-T. <i>Virchows Archiv B Cell Pathol.</i> 58: 129–145 (1989). Moll R, Franke W W and Schiller D L. <i>Cell.</i> 31: 11–24 (1982).
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# Instructions for Use

## High Temperature Antigen Unmasking Technique 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) 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. Wash sections in TBS\* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

### Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).

Add 3.84 g 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 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% 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, 10 mM 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 MANUFACTURER'S INSTRUCTIONS.