Novocastra™ Liquid Mouse Monoclonal Antibody Multi-Cytokeratin

Product Code: NCL-L-AE1/AE3

Intended Use	FOR RESEARCH USE ONLY.
Specificity	NCL-L-AE1/AE3 exhibits broad reactivity with two families of cytokeratin, acidic and basic, and may be used in the characterisation of cells of epithelial origin. Clone AE1 recognizes the 56.5, 50, 50', 48 and 40 kD human cytokeratins of the acidic subfamily. Clone AE3 recognizes the 65 to 67, 64, 59, 58, 56 and 52 kD human cytokeratins of the basic subfamily.
Clone	Cocktail of two clones, AE1 and AE3, mixed to a ratio of 20:1.
Ig Class	AE1, IgG1 AE3, IgG1
Antigen Used for Immunizations	Human epidermal cytokeratin preparation.
Hybridoma Partner	Mouse myeloma (P3X63Ag8).
Preparation	Liquid Protein A purified immunoglobulin diluted in PBS with 1% BSA containing 15 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Yes
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:20–1:100. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not evaluated.
Positive Controls	Immunohistochemistry: Normal skin.
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	Keratins are a family of water insoluble proteins of 40 to 70 kD. These proteins form tonofilaments, a class of intermediate filament, in epidermis as well as in almost all other epithelia. The process of normal epidermal differentiation is characterized by a series of morphological and biochemical changes as cells progress from the germinative basal layer through the spinous and granular layers to the outer cornified layer. The 65 to 67 kD cytokeratins are present only above the basal layer, the 58 kD cytokeratin is detected throughout the entire epidermis including the basal layer, the 56 kD cytokeratin is absent from the basal layer and first appears in the upper spinous layer. The 50 kD cytokeratin is the only major cytokeratin detected in the basal layer and is normally eliminated during stratum corneum formation. The 56 and 65 to 67 kD cytokeratins are characteristic of epidermal cells undergoing terminal differentiation and may be considered as molecular markers for keratinisation.
General References	Pinkus G S, O'Connor E M, Etheridge C L, et al.. The Journal of Histochemistry and Cytochemistry. 33 (5): 465–473 (1985). Weiss R A, Eichner R and Sun T–T. The Journal of Cell Biology. 98: 1397–1406 (1984). Tseng S C G, Jarvinen M J, Nelson W G, et al.. Cell. 30: 361–372 (1982). Woodcock–Mitchell J, Eichner R, Nelson W G, et al.. The Journal of Cell Biology. 95: 580–588 (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.