Novocastra™ Liquid Mouse Monoclonal Antibody Cytokeratin (5/6/8/18)

Product Code: NCL-L-CK5/6/8/18

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human cytokeratin 5, 6, 8 and 18 intermediate filament proteins. NCL-L-CK5/6/8/18 is a cocktail of monoclonal antibodies designed to recognize all epithelial tissue.
Clone	5D3 and LP34.
Ig Class	5D3, IgG1 LP34, IgG1
Antigen Used for Immunizations	5D3, Cytokeratins for the human breast carcinoma cell line MCF-7. LP34, Detergent-insoluble fraction of psoriatic human epidermis.
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
Effective on Paraffin Wax Embedded Tissue	Yes
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:50–1:100. Trypsin digestion of paraffin sections is recommended. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not evaluated.
Positive Controls	Immunohistochemistry: 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	Cytokeratins are a family of over 20 structurally related proteins that are differentially expressed in different epithelia and at various stages of differentiation and development. Cytokeratins belong to two families, type I (acidic) and type II (basic), which are usually co-ordinately synthesized in pairs so that at least one member of each family is expressed in each epithelial cell. Type I and type II cytokeratins form obligatory heterodimers that self assemble into 10 nm intermediate filaments. The antibody reacts with simple epithelium and both basal and suprabasal layers of cornifying and non-cornifying squamous epithelium.
General References	Hatzfeld M and Weber K. Journal of Cell Biology. 116: 157–166 (1992). Hatzfeld M and Weber K. Journal of Cell Science. 97: 317–324 (1990). Angus B, Kiberu S, Purvis J, et al.. Journal of Pathology. 155: 71–75 (1988). Angus B, Purvis J, Stock D, et al.. Journal of Pathology. 153: 377–384 (1987). Ghosh A K, Erber W N, Hatton C S R, et al.. British Journal of Haematology. 61: 21–30 (1985). Gatter K C, Abulaziz Z, Beverley P, et al.. Journal of Clinical Pathology. 35: 1253–1267 (1982). Moll R, Franke W W, Schiller D L, et al.. Cell. 31: 11–24 (1982).

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BIOSYSTEMS

Instructions for Use

Trypsin Digestion for Immunohistochemical Demonstration on Paraffin Sections

1. Preheat the following to 37 °C using a water bath:
   (i) 200 mL of TBS
   (ii) 200 mL of distilled water.
2. Dissolve 0.2 g Trypsin 250 and 0.2 g Calcium chloride in the 200 mL of TBS.
3. Once the Trypsin solution is at 37 °C, pH to 7.8 with 1 M sodium hydroxide.
4. Place rehydrated paraffin sections in the distilled water to preheat the sections to 37 °C for a minimum of 5 minutes.
5. Incubate sections in Trypsin solution at 37 °C. The time required will depend on the antibody and tissue, however, 30 minutes is usually sufficient.
6. Rinse sections in running tap water.
7. Proceed with immunohistochemistry protocol.

Reagents Required but not Supplied

50 mM Tris-buffered saline

Trypsin 250: Difco order code 0152–13 (available from Becton Dickinson).

Calcium chloride

1 M Sodium Hydroxide

** 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.*