

Novocastra™ Lyophilized Mouse Monoclonal Antibody Cytokeratin (8/18)

Product Code: NCL-5D3

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human cytokeratin 8 and 18 intermediate filament proteins.
Clone	5D3
Ig Class	IgG1
Antigen Used for Immunizations	Cytokeratins from the human breast carcinoma cell line MCF-7.
Hybridoma Partner	Mouse myeloma (p3-NS1-Ag4-1).
Preparation	Lyophilized tissue culture supernatant containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
Effective on Frozen Tissue	Yes
Effective on Paraffin Wax Embedded Tissue	Yes
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100. Trypsin digestion of paraffin sections is recommended. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not evaluated. Effective in indirect flow cytometry.
Positive Controls	Immunohistochemistry: Skin. Western Blotting: A549 cell line.
Staining Pattern	Cytoplasmic
Storage and Stability	Store unopened lyophilized 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. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.
General Overview	NCL-5D3 reacts with human cytokeratin intermediate filament proteins of 52.5 kD and 45 kD, identified as cytokeratins 8 and 18, respectively. In normal tissues, NCL-5D3 recognises all simple and glandular epithelium.
General References	Méhes G, Witt A, Kubista E, et al.. American Journal of Pathology. 159 (1): 17–20 (2001). Brotherick I, Robson C N, Browell D A, et al.. Cytometry. 32 (4): 301–308 (1998). Maurizii M G, Saverino O and Taddei C. Molecular Reproduction and Development. 48 (4): 536–542 (1997). 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).



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.*