

Novocastra™ Ready-to-Use Mouse monoclonal Antibody Cytokeratin (8/18)

Product Code: RTU-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	Tissue culture supernatant diluted in 5% horse serum in PBS containing 12 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Yes
Effective on Paraffin Wax Embedded Tissue	Yes, trypsin digestion of paraffin sections is recommended.
Recommendations on Use	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.
Positive Controls	Immunohistochemistry: Skin.
Staining Pattern	Cytoplasmic.
Storage and Stability	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.
General Overview	RTU-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, RTU-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.*