Novocastra™ Lyophilized Mouse Monoclonal Antibody Involucrin

Product Code: NCL-INV

Intended Use
FOR RESEARCH USE ONLY.

Specificity
Human involucrin. Crossreacts with pig and dog involucrin. No crossreaction with mouse involucrin.

Clone
SY5

Ig Class
IgG1

Antigen Used for Immunizations
Human involucrin (120 kD).

Hybridoma Partner
Mouse myeloma (X-63).

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 1:100–1:200. Trypsin digestion of sections may be required in some cases. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not recommended. Effective in immunoprecipitation techniques.

Positive Controls
Immunohistochemistry: Normal human skin.

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
Involucrin is a soluble cytoplasmic protein precursor of the epidermal cornified envelope that becomes cross-linked by transglutaminase during envelope assembly. Involucrin is expressed in a range of stratified squamous epithelia, including the cornea which lacks a distinct cornified layer. The protein is a useful marker of terminal differentiation. It is tightly linked to the onset of differentiation and first expressed in the immediate suprabasal layers of the epidermis.

General References
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.