Novocastra™ Liquid Mouse Monoclonal Antibody N-Cadherin

Product Code: NCL-L-N-Cad

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human N-Cadherin
Clone	IAR06
Ig Class	IgG2b
Antigen Used for Immunizations	Prokaryotic recombinant protein corresponding to 160 amino acids of the C-terminus of the human N-Cadherin molecule.
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	Not evaluated.
Effective on Paraffin Wax Embedded Tissue	Yes (using heat induced epitope retrieval with citrate-based buffer, pH 6.0: see overleaf)
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100. Heat induced epitope retrieval technique using Citrate-based buffer, pH 6.0. 30 minutes primary antibody incubation at 25 °C. Polymer detection recommended. Western Blotting: 1:250–1:1000 (ECL™, Amersham Pharmacia Biotech).
Positive Controls	Immunohistochemistry: Testis Western Blotting: PC3 cell line
Staining Pattern	Membrane and 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	N-Cadherin is a member of the cadherin family of calcium dependent cell adhesion molecules. The classical cadherins include the E, N, R, P and VE-Cadherins which are believed to expressed in a tissue specific manner. The classical cadherins have a characteristic structure comprising an extracellular calcium-binding domain, consisting of five cadherin repeats, a transmembrane domain and a highly conserved cytoplasmic domain, which mediates interactions with cytoskeletal components of the cell via interactions with intracellular proteins including the catenins. Cadherins play an important role in cell-cell adhesion, and are implicated in segregation and aggregation of tissues during development. N-Cadherin is reported to be expressed in various cell types including neural, myocardial and mesenchymal cells. During tumor progression increased N-Cadherin expression concomitant with the loss of E-Cadherin expression is one of the features of the epithelial to mesenchymal transition, which is associated with increased tumor invasion and poor prognosis.
General References	Agiostratidou G, Hulit J and Phillips et al. Journal of Mammary Gland Biology and Neoplasia. 12: 127–133 (2007). Mol A, Geldof A and Meijer G et al. Journal of Cancer Research Clinical Oncology. 133:687–695. (2007). Halbleib J and Nelson W. Genes & Development. 20:3199–3214 (2006).

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BIOSYSTEMS

Instructions for Use

Heat Induced Epitope Retrieval Combined With Polymer Detection 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 epitope retrieval solution (Citrate based pH 6.0 - Epitope Retrieval Solution unless otherwise indicated overleaf) in a stainless steel pressure cooker until boiling. 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 epitope retrieval 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 once using fresh Tris-Buffered Saline (TBS, pH 7.6) buffer for 5 minutes.
9. Place sections in diluted normal serum (eg NCL-G-SERUM) for 10 minutes.
10. Incubate sections with primary antibody.
11. Wash twice, each time using fresh TBS buffer for 5 minutes.
12. For visualization of the bound primary antibody, follow instructions supplied with the Polymer Detection System.
13. Counterstain with hematoxylin (if required), dehydrate and mount.

** (In most applications, Phosphate Buffered Saline, pH 7.6, can be used instead of TBS, pH 7.6).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ THE MANUFACTURER'S INSTRUCTIONS.

protocol/RUO/HIER+Polymer/04/08