Novocastra™ Liquid Mouse Monoclonal Antibody *Helicobacter pylori*

Product Code: NCL-L-Hpylori

Intended Use	FOR RESEARCH USE ONLY.
Specificity	*Helicobacter pylori*
Clone	ULC3R
Ig Class	IgG1
Antigen Used for Immunizations	Prokaryotic recombinant protein corresponding to 151 amino acids of the HopE 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 with citrate–based buffer, pH 6.0. 30 minutes primary antibody incubation at 25 °C. Polymer detection recommended. Western Blotting: Typical working dilution: 1:250–1:1000 (ECL™, Amersham Pharmacia Biotech).
Positive Controls	Immunohistochemistry: *Helicobacter pylori* infected tissue. Western Blotting: *Helicobacter pylori* lysate.
Staining Pattern	*Helicobacter pylori* bacteria.
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	*Helicobacter pylori* is a motile, helix–shaped Gram–negative, microaerophilic, bacterial pathogen which is capable of converting from a spiral form to a coccoid form to favor its survival. Almost 50 % of the world's population, approaching 100 % in some countries, are infected. There are numerous strains of *Helicobacter pylori* which can be grouped into two broad families, type I and type II, based on their expression of the hopQ allele. Type I and type II strains are reported to express VacA (vacuolating toxin) responsible for vacuolation of gastric epithelial cells and induction of apoptosis. Type I strains are reported to express CagA protein which is associated with deregulation of intercellular signalling pathways and initiation of pathogenesis (virulent strains) and are closely related to gastric diseases such as duodenal and gastric ulceration, chronic gastritis, mucosa–associated lymphoid tissue (MALT) lymphoma and intestinal type gastric adenocarcinomas. Type II strains are reported not to express CagA proteins. HopE is a 31 kD porin protein which is part of a family of 32 outer membrane proteins present in *Helicobacter pylori* bacteria. HopE is highly conserved in *Helicobacter pylori* strains, but not among other strains of the *Helicobacter* genus.
General References	Cao P and Cover T. Journal of Clinical Microbiology. 2002; 40(12):4504–4511. Doig P, Exner M, Hancock R et al. Journal of Bacteriology. 1995; 177(19):5447–5452.

Leica Biosystems Newcastle Ltd
Balliol Business Park West
Benton Lane
Newcastle Upon Tyne NE12 8EW
United Kingdom
✆ +44 191 215 4242

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.