Novocastra™ Liquid Mouse Monoclonal Antibody Mismatch Repair Protein 6

Product Code: NCL-L-MSH6

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human mismatch repair protein 6 (MSH6)
Clone	PU29
Ig Class	IgG1
Antigen Used for Immunizations	Prokaryotic recombinant protein corresponding to a 359 amino acid portion of the human MSH6 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, pH6.0: 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. Technical Note: The use of PBS-based diluents may result in increased background staining. Western Blotting: 1:500–1:2000 (ECL™, Amersham Pharmacia Biotech).
Positive Controls	Immunohistochemistry: Bowel Western Blotting: A431 cell line
Staining Pattern	Nuclear
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	MSH6 is a 160 KDa protein which is involved in DNA mismatch repair (MMR) and recombination pathways, when heterodimerized with MSH2. Defects in mismatch repair systems can cause mutations and can cause DNA microsatellite sequences to become unstable. Microsatellite instability has been described in colorectal cancer, particularly in Hereditary Nonpolyposis Colorectal Cancer (HNPCC) where MSH6 expression, along with other MSH proteins, is disrupted. Immunohistochemical studies have reported that MSH6 is strongly expressed in the nucleus of cells in normal colonic epithelium, especially in crypts. Expression is also found in lymphocytes. Studies have also shown that MSH6 is expressed in gastric carcinomas and endometrial carcinomas. However, sometimes expression can be lost in some endometrial carcinomas and colonic carcinomas with microsatellite instability. MSH6 has been reported to be a useful marker to use in conjunction with microsatellite instability screening to identify colon tumors that may contain MMR gene mutations, such as HNPCC.
General References	Cederquist K, Emanuelsson M, Goransson I et al. International Journal of Cancer.109(3):370–376 (2004). Schweizer P, Moisio AL, Kuismanen SA et al. Cancer Research. 61, 2813–2815 (2001). Semba S, Ouyang H, Han SY et al. International Journal of Oncology.16(4):731–737 (2000). Parc YR, Halling KC, Wang L et al. Cancer Research. 60, 2225–2231 (2000).

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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.