Novocastra™ Liquid Mouse Monoclonal Antibody Mismatch Repair Protein 6 (MSH6)

Product Code: NCL-L-MSH6

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

EN

Instructions for Use

Please read before using this product.

Check the integrity of the packaging before use.

Novocastra™ Liquid Mouse Monoclonal Antibody Mismatch Repair Protein 6 (MSH6)

Product Code: NCL-L-MSH6

Intended Use

For in vitro diagnostic use.
NCL-L-MSH6 is intended for the qualitative identification by light microscopy of Mismatch Repair Protein 6 (MSH6) molecules in paraffin sections. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Principle of Procedure

Immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody and an enzyme complex with a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Clone

PU29

Immunogen

Prokaryotic recombinant protein corresponding to a 359 amino acid portion of the MSH6 human molecule.

Specificity

Human mismatch repair protein 6

Reagent Composition

NCL-L-MSH6 is a liquid tissue culture supernatant containing 15 mM sodium azide as a preservative.

Ig Class

IgG1

Total Protein Concentration Total Protein

1.0 - 8.0. g/L. Refer to vial label for lot specific total protein concentration.

Antibody Concentration

Greater than or equal to 200 mg/L as determined by ELISA. Refer to vial label for batch specific Ig concentration.

Recommendations On Use

Immunohistochemistry (see **Methodology**) on paraffin sections. Suggested dilution: 1:100 for 30 minutes at 25 °C. Heat induced epitope retrieval using Epitope Retrieval Solution pH6.0 RE7113, RE7114 or RE7115. This is provided as a guide and users should determine their own optimal working dilutions. Technical note: The use of PBS-based diluents may result in increased background staining

Storage and Stability

Store at 2–8 °C. Do not freeze. Return to 2–8 °C immediately after use. Do not use after expiration date indicated on the vial label. Storage conditions other than those specified above must be verified by the user.

Specimen Preparation

The recommended fixative is 10% neutral-buffered formalin for paraffin-embedded tissue sections.

Warnings and Precautions

This reagent has been prepared from the supernatant of cell culture. As it is a biological product, reasonable care should be taken when handling it.
The molarity of sodium azide in this reagent is 15 mM. A Material Safety Data Sheet (MSDS) is available upon request for sodium azide. Consult federal, state or local regulations for disposal of any potentially toxic components.
Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.[1] Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.
Minimize microbial contamination of reagents or an increase in non-specific staining may occur.
Incubation times or temperatures, other than those specified, may give erroneous results. Any such changes must be validated by the user.

Quality Control

Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the following procedures.
Controls should be fresh autopsy/biopsy/surgical specimens, formalin-fixed, processed and paraffin wax-embedded as soon as possible in the same manner as the patient sample(s).

Positive Tissue Control

Used to indicate correctly prepared tissues and proper staining techniques.
One positive tissue control should be included for each set of test conditions in each staining run.
A tissue with weak positive staining is more suitable than a tissue with strong positive staining for optimal quality control and to detect minor levels of reagent degradation.[2]

Recommended positive control tissue is colon.
If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control

Should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody.
Recommended negative control tissue is skeletal muscle.
Alternatively, the variety of different cell types present in most tissue sections frequently offers negative control sites, but this should be verified by the user.
Non-specific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain non-specifically.[3] False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by endogenous enzymes such as pseudoperoxidase (erythrocytes), endogenous peroxidase (cytochrome C), or endogenous biotin (eg. liver, breast, brain, kidney) depending on the type of immunostain used. To differentiate endogenous enzyme activity or non-specific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate chromogen or enzyme complexes (avidin-biotin, streptavidin, labeled polymer) and substrate-chromogen, respectively. If specific staining occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Negative Reagent Control

Use a non-specific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate non-specific staining and allow better interpretation of specific staining at the antigen site.

Patient Tissue

Examine patient specimens stained with NCL-L-MSH6 last. Positive staining intensity should be assessed within the context of any non-specific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

Results Expected

Normal Tissues
Clone PU29 detected the MSH6 protein expressed in the nuclei of cells in a variety of tissues (n=69). Staining was observed in colon, small bowel, appendix, adrenal, lung, stomach, esophagus, skin, ovary, cervix, prostate, thymus, salivary gland, thyroid, parathyroid, breast, pituitary, tonsil, bone marrow, gall bladder, placenta and spinal cord.

Tumor Tissues
Clone PU29 detected the MSH6 protein in 54/58 colon tumors. Clone PU29 also detected the MSH6 protein in lymphomas (9/11), sarcomas (7/7), ovarian tumors (7/7), lung tumors (6/7), breast tumors (4/5), stomach tumors (4/4), endometrial tumors (4/4), neuroendocrine tumors (2/4), BPH/prostate tumor (3/3), melanomas (3/3), adrenal tumors (3/3), germ cell tumors (3/3), bladder tumors (3/3), squamous cell carcinomas (2/3), liver tumors (1/3), pancreatic tumors (2/2), thyroid tumors (2/2), small bowel tumor (1/1), skin tumor (1/1), thymus tumor (1/1), neural tumor (0/1) and kidney tumors (0/4) (n=140).

NCL-L-MSH6 is recommended for the detection of the MSH6 protein in normal and neoplastic tissues, as an adjunct to conventional histopathology using non-immunologic histochemical stains.

General Limitations

Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.[4]
Excessive or incomplete counterstaining may compromise proper interpretation of results.
The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.
Antibodies from Leica Biosystems Newcastle Ltd are for use, as indicated, on either frozen or paraffin-embedded sections with specific fixation requirements. Unexpected antigen expression may occur, especially in neoplasms. The clinical interpretation of any stained tissue section must include morphological analysis and the evaluation of appropriate controls.

Bibliography - General

1. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, P.A. 1991; 7(9). Order code M29-P.
2. Battifora H. Diagnostic uses of antibodies to keratins: a review and immunohistochemical comparison of seven monoclonal and three polyclonal antibodies. Progress in Surgical Pathology. 6:1–15. eds. Fenoglio-Preiser C, Wolff CM, Rilke F. Field & Wood, Inc., Philadelphia.
3. Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and pitfalls. Laboratory Medicine. 1983; 14:767.
4. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen: a possible source of error in immunohistochemistry. American Journal of Clinical Pathology. 1980; 73:626.
5. Warren J, Pohlhaus T, Changela A, et al. Structure of the human MutSα DNA lesion recognition complex. Molecular Cell. 2007; 26:579-592.
6. Marti T, Kunz C, Fleck O, et al. DNA mismatch repair and mutation avoidance pathways. Journal of Cellular Physiology. 2002; 191:28-41.

Amendments to Previous Issue

Results Expected.

Date of Issue

22 September 2020

Immunohistochemical Methodology For Novocastra™ Antibodies On Paraffin-Embedded Tissue Utilizing The Heat Induced Epitope Retrieval Technique.

Reagents Required but not Supplied

1. Standard solvents used in immunohistochemistry.
2. 50 mM Tris-Buffered Saline (TBS) pH 7.6.
3. Epitope Retrieval Solution (see C. Epitope Retrieval Solutions).
4. Antibody diluent, Novocastra IHC Diluent, RE7133.
5. Visualization system, Novolink™ Polymer Detection Systems, RE7280-K (1250 tests), RE7150-K (500 tests), RE7140-K (250 tests) or RE7290-K (50 tests).
6. Mounting medium - use as recommended by manufacturer.

Equipment Required but not Supplied

1. Incubator set to 25 °C.
2. Heating device for epitope retrieval: water bath, steamer, pressure cooker or other temperature controlled laboratory equipment.
3. General immunohistochemistry laboratory equipment.

Epitope Retrieval Solutions (see Recommendations on Use for one of the following)

RE7113 Epitope Retrieval Solution pH 6 (x10 Concentrate) 1 L	Citrate-based buffer containing surfactant
RE7114 Epitope Retrieval Solution pH 6 (x10 Concentrate) 500 mL	
RE7115 Epitope Retrieval Solution pH 6 (Ready to Use) 1 L	
RE7116 Epitope Retrieval Solution pH 8 (x10 Concentrate) 1 L	EDTA-based buffer containing surfactant
RE7118 Epitope Retrieval Solution pH 8 (Ready to Use) 1 L	
RE7119 Epitope Retrieval Solution pH 9 (x10 Concentrate) 1 L	Tris/EDTA-based buffer containing surfactant
RE7122 Epitope Retrieval Solution pH 9 (Ready to Use) 1 L	

Methodology

Prior to undertaking this methodology, users must be trained in immunohistochemical techniques.

Users should determine optimal dilutions for antibodies. Unless indicated, all steps are performed at room temperature (25 °C).

Epitope Retrieval

Please follow the instructions for use in Epitope Retrieval Solutions, RE7113, RE7114, RE7115, RE7116, RE7118, RE7119 or RE7122.

Visualization

Please follow the instructions for use in the Novolink Polymer Detection Systems, RE7280-K (1250 tests), RE7150-K (500 tests), RE7140-K (250 tests) or RE7290-K (50 tests).

Amendments to Previous Issue

Not applicable

Date of Issue

23 April 2008 (CEprotocol/HTAUT+Novolink).

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

Leica Biosystems Canada
71 Four Valley Drive
Concord, Ontario L4K 4V8
Canada
✆ +1 800 248 0123

Leica Biosystems Inc
1700 Leider Lane
Buffalo Grove IL 60089
USA
✆ +1 800 248 0123

Leica Biosystems Melbourne
Pty Ltd
495 Blackburn Road
Mt Waverley VIC 3149
Australia
✆ +61 2 8870 3500