

Anti-MLH-1 (Clone: G168-15)

Mouse monoclonal antibody

Reference: AP10277; AP10277C



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INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.

AP10277, 7 ml. Prediluted antibodies in a synthetic organic linear polymer buffer solution (pH 7.4), with carrier protein and preservative for stabilisation "READY TO USE"

AP10277C, 1 ml. Concentrated antibodies with carrier protein and preservative for stabilisation.

SUMMARY, EXPLANATION AND LIMITATIONS:

The repair of mismatch DNA is essential to maintaining the integrity of genetic information over time. Defects on the MMR (mismatched repair) system are involved in tumorous progression of sporadic human neoplasms, familial or hereditary in nature. In yeasts, proteins are denominated MutS homologous 2 (MSH2), MutL homologous (MLH) and PMS1, which is also homologous to MutL. MSH2 is considered to be involved in mismatched nucleotides initial recognition during MMR process. MSH2 binds to DNA altered duplex and later it binds to a complex formed by MSH1 and PMS1 heterodimer, the whole complex facilitates the DNA final repair. Those mechanisms are also proposed for MMR in human. DNA mismatched repair genes are involved in the development of a group of colorectal, gastric, and sporadic endometrial tumors. Microsatellites instability (MSI) or expression loss has been detected in 13-44% of gastric neoplasms, in 10-15% of colorectal neoplasms and in 17-23% of sporadic endometrial neoplasms. In these cases, MSI phenotype is consistent with a MMR gene somatic defect. MLH1 is transcriptionally inhibited in 90% of sporadic cancers, although in a minority of cases it shows a MLH1 or MSH2 inactivation due to a somatic mutation.

Immunohistochemistry (IHC) is a complex technique in which immunological and histological detection methods are combined. In general, the manipulation and processing of tissues before immunostaining, especially different types of tissue fixation and embedding, as well as the nature of the tissues themselves may cause inconsistent results (Nadji and Morales, 1983). Endogenous pseudoperoxidase and peroxidase activity or endogenous biotin and alkaline phosphatase activity can cause non-specific staining results depending on the detection system used. Tissues that contain Hepatitis B surface antigen (HBsAg) can produce false positives when using HRP detection systems (Omata et al, 1980). Insufficient contrast staining and/or improper mounting of the sample may influence the interpretation of results.

Isotype: IgG1 Kappa

Immunogen: Recombinant MLH-1 protein.

Staining pattern: Mainly nuclear, light cytoplasmic stain can be observed.

The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.

Positive control: Tissue sample from tonsil or colon.

External negative control: Tissue sample homologous to the

test sample incubated with an antibody isotype not specific for MLH-1.

APPLICATIONS:

This antibody is designed for the specific localization of human MLH-1 using IHC techniques in formalin-fixed, paraffin-embedded tissue sections.

Microsatellite instability or the absence of expression of proteins involved in DNA repair (MLH-1 and MSH-2) are attributed a prognostic value in colorectal sporadic, gastric and endometrial neoplasms.

PRODUCT COMPOSITION:

Mouse immunoglobulin IgG1 Kappa, clone G168-15, obtained from culture supernatant purified by affinity chromatography. The preparation contains saline buffer, stabilising and carriers proteins, and sodium azide as a preservative.

METHODS AND PROCEDURE:

Principles of the procedure: The demonstrations of antigens by IHC is a sequential procedure with several steps involving first the application of a specific antibody for the antigen of interest (primary antibody), then a secondary antibody which joins to the first, an enzyme complex, and the addition of a chromogenic substrate. The sample is washed between each step. Enzymatic activation of the chromogenic substrate creates a visible product where the antigen is located. The results are interpreted using a light microscope. The primary antibody can be used both in manual IHC and with automated immunostainers.

Specimen: Paraffin-embedded tissue samples should be used. Western blot techniques are not recommended.

Staining procedure:

Antigen retrieval	HIER Citrate Buffer pH 6.5
Working dilution (only for concentrates)	1:25 – 1:100 with <i>Antibody Diluent S Yellow</i> (AP12001)
Incubation	30 min; RT
Control Tissue	Tonsil or colon

Amplification and development of the immunostaining:

Follow standard procedure and the recommendations given by the manufacturer for the materials used. In the case of using automated immunostainers, use the specified buffers and materials for each instrument.

See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for IHC procedures are not provided with this antibody. This includes adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H₂O, heat pretreatment equipment (pressure cooker, steamer, microwave), pipettes, Coplin jars, glass jars, moist chamber, histological baths, negative control reagents, counter-staining



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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solution, mounting materials, and microscope.

Buffered solutions for antigen retrieval, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Genova Scientific.

STORAGE AND STABILITY:

Store at 2-8 °C until the expiration date printed on product label. Do not use after the expiration date. If fresh solutions are required, these must be prepared immediately prior to use, and will be stable for at least one day at room temperature (20-25°C). Unused portion of antibody preparation should be discarded after one day. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user. The validity period of the ready to use products when opened, is the same as the expiration date indicated on the label of intact product.

Genova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances is Genova Scientific obliged to cover damages caused by use of this reagent.

TROUBLESHOOTING:

If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the detection system. If this does not solve the problem, please contact Genova Scientific's technical support department or your local distributor.

PRECAUTIONS:

Use only by qualified personnel.

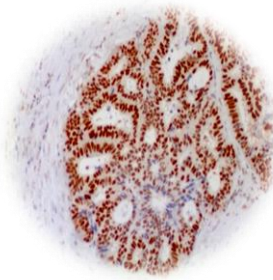
Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific staining results. This antibody contains sodium azide (NaN₃), used as a stabilising agent, which is not considered to be a hazardous material in the concentration used. Concentration of sodium azide in drainage pipes made of lead or copper can cause the formation of highly explosive metallic azides. In order to avoid this, sodium azide must be disposed of along with a large volume of running water. Material safety data sheet (MSDS) for pure sodium azide is available upon request.

PERFORMANCE CHARACTERISTICS:

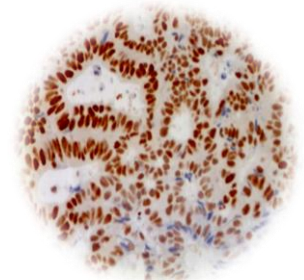
Genova Scientific has performed studies to evaluate the functioning of these antibodies for use with standard detection systems, concluding that the product is both specific and sensitive for the antigen of interest.

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Nadji M, Morales AR. Immunoperoxidase, part 1: the techniques and its pitfall. Lab Med 1983; 14:767-770.
Omata M, Liew CT, Ashcavai M, Peters RI. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol. May, 1980;73(5):626-632.



10 ×



20 ×

IHC of MLH-1 (clone G168-15) on FFPE colon adenocarcinoma tissue.

Citrate pH 6.5; DAB; Hematoxylin

F01IT04_V3R0712_AP10277_English



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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