anti-MLH-1 (M1) Mouse Monoclonal Primary Antibody

REF 790-4535

06472966001

IVD Σ 50

INTENDED USE

Anti-MLH-1 (M1) Mouse Monoclonal Primary Antibody (anti-MLH-1 (M1)) is used to qualitatively identify human DNA mismatch repair (MMR) protein MLH1, expressed in the nucleus of normal proliferating cells. Deficient or low levels of MLH1 are associated with colorectal and other cancers. The antibody is intended for qualitative staining in sections of formalin-fixed, paraffin-embedded tissue.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for *in vitro* diagnostic (IVD) use.

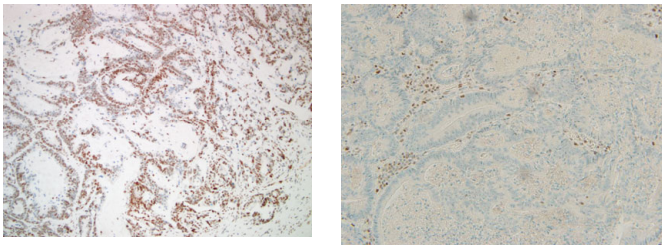

Figure 1. Anti-MLH-1 (M1) staining of colon adenocarcinomas from two different specimens. Left panel: MLH1-positive cells and cells with loss of MLH1. Right panel: HNPCC case with complete loss of MLH1 in epithelial cells (stromal cells are MLH1-positive).

SUMMARY AND EXPLANATION

Anti-MLH-1 (M1) is a mouse monoclonal antibody produced against a full-length recombinant MLH1 protein with a GST tag. Anti-MLH-1 (M1) recognizes MLH1, which is one of several clinically important mismatch repair (MMR) proteins that are mutated in families with hereditary non-polyposis colorectal cancer (HNPCC).[1,2] Carriers of these mutations have a high lifetime risk of developing colorectal and several other cancers due to accumulation of DNA replication errors in proliferating cells, a phenomenon known as microsatellite instability (MSI). In normal cells, the MLH1 protein forms complexes (heterodimers) with PMS2 protein.[3] When DNA mismatches occur, heterodimers of other MMR proteins, MSH6 and MSH2, bind to the mismatched DNA, inducing conformational changes. The MLH1/PMS2 complexes bind the DNA-bound MSH6/MSH2 complexes resulting in excision repair of the affected DNA. Mutations or deficiencies in these proteins result in frequent MSI and somatic mutation due to replication error.

HNPCC represents 1-3% of all colorectal cancers, with MLH1 loss occurring in the majority of these.[4] More than 300 different mutations in the MMR family of proteins have been identified in people with HNPCC. The HNPPC-associated tumor phenotype is generally characterized by immunohistochemical loss of expression in MMR proteins, particularly MLH1, MSH2, MSH6 and PMS2.[5,6] Deficiencies in these proteins are closely related to the degree of MSI in a tumor. MMR protein deficiencies are thus used in classification of tumors as MSI-H (high degree of MSI), MSI-L (low degree of MSI), or MSS (MS stable).[7] Each classification has implications in treatment and prognosis of the tumor. Patients classified as MSI-H, despite having a much higher likelihood of developing cancer, also have a significant survival advantage, independent of stage or grade, over patients with MSS, which is typically characterized by chromosomal instability.

REAGENT PROVIDED

Anti-MLH-1 (M1) contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-MLH-1 (M1) contains approximately 7.5 µg of a mouse monoclonal antibody.

The antibody is diluted in a TBS buffer containing 0.3% carrier protein.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 1.4 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Anti-MLH-1 (M1) is a recombinant mouse monoclonal antibody produced as cell culture supernatant.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as Ventana detection kits (*ultra*View Universal DAB Detection Kit), and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana BenchMark Series automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[8] Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents as it may cause incorrect results.
5. Consult local and/or state authorities with regard to recommended method of disposal.
6. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at www.ventana.com.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana BenchMark Series automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark XT instrument and BenchMark ULTRA instrument with *ultra*View Universal DAB Detection Kit is listed in Table 1.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate Ventana detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for anti-MLH-1 (M1) with *ultra*View Universal DAB Detection Kit on a BenchMark XT instrument and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1 Standard
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument 16 Minutes, 37°C BenchMark ULTRA instrument 16 Minutes, 36°C
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

Table 2. Recommended Staining Protocol for anti-MLH-1 (M1) with *i*VIEW DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1 Standard
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument 32 Minutes, 37°C
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[9]

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are normal proliferating cells of colon, breast, appendix, testis and ovary.

STAINING INTERPRETATION

The cellular staining pattern for anti-MLH-1 (M1) is nuclear. Homogeneous chromatin staining is interpreted as positive. Discreet, punctate nuclear staining is interpreted as negative. Staining along the nuclear membrane is interpreted as negative.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16-minute incubation time on a BenchMark XT instrument and BenchMark ULTRA instrument in combination with *ultra*View Universal DAB Detection Kit but the user must validate results obtained with this reagent.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and reproducibility were conducted using anti-MLH-1 (M1) with *ultra*View Universal DAB Detection Kit on BenchMark XT and BenchMark ULTRA instruments.

Specificity

Table 3. Specificity of anti-MLH-1 (M1) was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	3/3
Cerebellum	2/3	Myeloid (bone marrow)	3/3
Adrenal gland	1/3	Mesothelium and lung	2/6
Ovary	3/3	Heart	1/3
Pancreas	2/2	Esophagus	1/3
Thyroid	3/3	Small intestine	1/3
Parathyroid gland	3/3	Colon	51/57
Hypophysis	3/3	Salivary gland	1/3
Testis	3/3	Kidney	1/3
Breast	3/3	Prostate	1/3
Spleen	3/3	Cervix	3/3
Tonsil	3/3	Skin	3/3
Endometrium	1/3	Rectum	3/4
Skeletal muscle	3/3		
Nerve	0/3		
Lymph node	5/8		

Sensitivity

Table 4. Sensitivity of anti-MLH-1 (M1) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	1/1
Atypical meningioma	1/1
Malignant ependymoma	1/1
Malignant oligodendroglioma	1/1
Serous papillary adenocarcinoma	1/1
Mucinous papillary adenocarcinoma	1/1
Islet cell carcinoma	1/1
Pancreatic adenocarcinoma	1/1
Seminoma	1/1
Embryonal carcinoma	1/1
Medullary carcinoma	1/1
Papillary carcinoma	1/1
Breast intraductal carcinoma	2/2
Breast invasive ductal carcinoma	1/1
Diffuse B-cell lymphoma	3/3

Pathology	# positive / total cases
Lung small cell undifferentiated carcinoma	1/1
Lung squamous cell carcinoma	1/1
Lung adenocarcinoma	1/1
Esophageal squamous cell carcinoma	1/1
Esophageal adenocarcinoma	1/1
Gastric mucinous adenocarcinoma	1/1
Colorectal mucinous adenocarcinoma	19/21
Colorectal adenocarcinoma	84/ 108
Colorectal papillary adenocarcinoma	11/13
Colorectal adenosquamous carcinoma	2/2
Colorectal neuroendocrine carcinoma	2/2
Colorectal squamous cell carcinoma	4/5
Gastrointestinal intermediate grade malignant interstitialoma	2/2
Rectal adenocarcinoma	1/3
Rectal interstitialoma	1/1
Hepatocellular carcinoma	1/1
Hepatoblastoma	1/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	1/1
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Uterine clear cell carcinoma	1/1
Uterine squamous cell carcinoma	2/2
Embryonal rhabdomyosarcoma	1/1
Smooth muscle spindle cell rhabdomyosarcoma	1/1
Rectal malignant melanoma	1/1
Basal cell carcinoma	1/1
Skin squamous cell carcinoma	1/1
Neurofibroma	1/1
Ganglioneuroblastoma	1/1
Malignant mesothelioma	1/1
Hodgkin lymphoma	1/1
Diffuse malignant lymphoma	1/1
Transitional cell carcinoma	1/1
Leiomyosarcoma	4/4
Osteosarcoma	1/1
Signet-Ring Cell Carcinoma	1/1
Hereditary Non-polyposis Colorectal carcinoma (HNPCC)	0/14

Reproducibility

Reproducibility studies for anti-MLH-1 (M1) were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark XT instrument.
- Intra-platform reproducibility on the BenchMark XT instrument and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark XT instrument and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

REFERENCES

1. Boyer J, Umar A, Risinger J, Lipford J, Kane M, Yin S, Barrett C, Kolodner R, Kunkel T. Microsatellite instability, mismatch repair deficiency, and genetic defects in human cancer cell lines. Cancer Res. 1995;55:6063-6070.
2. Lawes, D, Pearson T, SenGupta S, Boulos P. The role of MLH1, MSH2 and MSH6 in the development of multiple colorectal cancers. Br J Cancer. 2005;93:472-477.
3. Chen Y, Wand J, Fraig MM, Metcalf J, Turner WR, Bissada NK, Watson DK, Schweinfest CW. Defects of DNA mismatch repair in human prostate cancer. Cancer Res. 2001;61(10):4112-4121.
4. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, Chadwick RB, Kaariainen H, Eskelinen M, Jarvinen H, Mecklin JP, de la Chapelle A: Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med. 1998;338:1481-1487.
5. Caldés, T. Immunohistochemistry and microsatellite instability testing for selecting MLH1, MSH2 and MSH6 mutation carriers in hereditary non-polyposis colorectal cancer. Oncol. Rep. 2004;12:621-629.
6. Ellis NA. Value of Immunohistochemical Detection of DNA Mismatch Repair Proteins in Predicting Germline Mutation in Hereditary Colorectal Neoplasms. Am J Surg Pathol. 2005 Jan 29(1):96-104.
7. Berends MJW, Wu Y, Sijmons RH, Mensink RGJ, van der Sluis T, Hordiik-Hos JM, deVries EGE, Hollema H, Karrenbeld A, Buys CH, van der Zee AGJ, Hofstra RMW, Kleibeuker JH. Molecular and clinical characteristics of *MSH6* variants: an analysis of 25 index carriers of a germline variant. Am J Hum Genet. 2002;70:26-37.
8. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
9. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.

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