MLH-1

Concentrated and Prediluted Monoclonal Antibody
901-220-011620

BIOCARE MEDICAL

Catalog Number:	CM 220 AK, BK, CK	PM 220 AA, H	IPI 220 G10	OAI 220 T60	AVI 220 G
Description:	0.1, 0.5, 1.0 mL, conc.	6.0, 25 mL, RTU	10 mL, RTU	60 tests, RTU	6.0 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Van Gogh Yellow	N/A	N/A	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
MLH-1 [G168-15] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of MLH-1 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. 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.

Summary and Explanation:
The G168-15 antibody recognizes human and mouse MLH-1 (80-85 kDa). The repair of mismatch DNA is essential to maintaining the integrity of genetic information over time. An alteration of microsatellite repeats is the result of slippage owing to strand misalignment during DNA replication and is referred to as microsatellite instability (MSI) (1-3). These defects in DNA repair pathways have been related to human carcinogenesis. The importance of mismatch repair genes became apparent with the identification of the genetic basis for hereditary nonpolyposis colon cancer (HNPC) (1-3). MSH-2 is involved in the initial cognition of mismatch nucleotides during the replication mismatch repair process. It is thought that after MSH-2 binds to a mismatched DNA duplex it is joined by a heterodimer of MLH-1 and PMS2, which together help facilitate the later steps in mismatch repair (1-3). Patients with colorectal carcinoma that is mismatch-repair-deficient and confirmed with immunohistochemistry (IHC) (MSH2/MSH6 negative or MLH1/PMS2 deleted) have shown objective response to PD-1 antibody, pembrolizumab (4). PD-L1 IHC test has been demonstrated to be a useful predictive marker for anti-PD-1 immunotherapy in colorectal carcinoma (5).

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human, mouse and rat
Clone: G168-15
Isotype: IgG1/kappa
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: MLH-1
Cellular Localization: Nuclear
Positive Tissue Control: Colon cancer
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative
Van Gogh Yellow (PD902)

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB -OR- Incubate for 5-7 minutes at RT with Warp Red.
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
intelliPATH FLX Automated Slide Stainer:
IPI220 is intended for use with the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.
Technical Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (ONCORE™ Automated Slide Staining System):
OAI220 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: MLH-1
Protocol Template (Description): IHC Extras Template
Dewaxing (DS Option): DS2
Antigen Retrieval (AR Option): AR1, high pH; 103°C
Reagent Name, Time, Temp.: MLH-1, 30 min., 25°C
- Use of **Mouse Amp HRP Detection (ORI6050) is required** for the above antibody protocol. Mouse HRP Detection (ORI6007) is not recommended.

Protocol Recommendations (Ventana BenchMark ULTRA):
AVI220 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Template/Detection: OptiView DAB
Pretreatment Protocol: CC2 48 minutes, 100°C
Peroxidase: Pre Primary Peroxidase Inhibitor
Option (V-Blocker BRI4001): Incubate for 4 minutes (with appropriate Option # registered by user)
V-Blocker is recommended to be applied prior to any primary antibody.
Primary Antibody: 60 minutes, 37°C

Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

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Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011.

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)
2. 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. 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. (7)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Vilkin, A, *et al*. Immunohistochemistry staining for mismatch repair proteins: the endoscopic biopsy material provides useful and coherent results. Hum Pathol 2015;46:1705–11.
2. Djordjevic B, Broaddus RR. Laboratory assays in evaluation of lynch syndrome in patients with endometrial carcinoma. Surg Pathol Clin 2016;9:289-99.
3. Peiro G, *et al*. Prognostic relevance of hMLH1, hMSH2, and BAX protein expression in endometrial carcinoma. Mod Pathol. 2001 Aug;14(8):777-83.
4. Lee LH, *et al*. Patterns and prognostic relevance of PD-1 and PD-L1 expression in colorectal carcinoma. Mod Pathol. 2016;29:1333-42.
5. Le DI, *et al*. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372:2509-20.
6. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
7. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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