

MLH-1

Concentrated and Prediluted Monoclonal Antibody
901-220-032222

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M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 220 AK, BK, CK	0.1, 0.5, 1.0 mL	1:100	Van Gogh Yellow
Predilute	PM 220 AA, H	6.0, 25 mL	Ready-to-use	N/A
intelliPATH FLX	IPI 220 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 220 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 220 T60	60 tests	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 220 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 220 G7	7.0 mL	Ready-to-use	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. 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).

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-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind 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 2°C to 8°C. 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 2°C to 8°C.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 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 (ONCORE™ Pro Automated Slide Staining System):

OPAI220 is intended for use with the ONCORE Pro. 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 1

Dewaxing (DS Buffer Option): DS2-50

Antigen Retrieval (AR Option): AR1, high pH; 103°C

Block Option: Buffer

Reagent Name, Time, Temp.: MLH-1, 30 min., 25°C

- Use of **Mouse Amp HRP Detection (OPRI6050)** is required for the above antibody protocol. Mouse HRP Detection (OPRI6007) is not recommended.

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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

Protocol Recommendations (Q Series – For Leica BOND-III):

ALI220 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Protocol Name: IHC Protocol F + Blocker

Detection: Bond Polymer Refine

HIER: 30 min with ER2

Peroxide Block: 5 min

Background Block: 10 min

Marker (Primary Antibody): 15 min

Post Primary: 8 min

Polymer: 8 min

Mixed DAB Refine: 10 min

Hematoxylin: 5 min

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.

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 (NaN₃) 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) (4)
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. (5)
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. 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."
5. 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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