

MLH-1

Concentrated and Prediluted Monoclonal Antibody
902-220-032222

BIOCARE
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACR 220 AK, BK, CK	0.1, 0.5, 1.0 mL	1:100	Van Gogh Yellow
Predilute	APR 220 AA, H	6.0, 25 mL	Ready-to-use	N/A
Q Series— For Leica BOND-III	ALR 220 G7	7.0 mL	Ready-to-use	N/A

Intended Use:

For Research Use Only. Not for use in diagnostic procedures.

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 (BRR902)

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.

Staining 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 Bluig Solution for 1 minute. Rinse with deionized water.

Staining Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:

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 (Q Series – For Leica BOND-III):

ALR220 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:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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>.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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.



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References Cont'd:

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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