

## MLH-1

Concentrated and Prediluted Monoclonal Antibody  
902-220-010418

**BIOCARE**  
M E D I C A L

<b>Catalog Number:</b>	<b>ACR 220 AK, BK, CK</b>	<b>APR 220 AA, H</b>
<b>Description:</b>	0.1, 0.5, 1.0 ml, concentrated	6.0, 25 ml, prediluted
<b>Dilution:</b>	1:100	Ready-to-use
<b>Diluent:</b>	Van Gogh Yellow	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. The importance of mismatch repair genes became apparent with the identification of the genetic basis for hereditary nonpolyposis colon cancer (HNPCC) (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. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human, mouse and rat

**Clone:** G168-15

**Isotype:** IgG1/kappa

**Total Protein Concentration:** ~10 mg/ml. 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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

### Staining Protocol Recommendations:

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Biocare's 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 Biocare's Warp Red.

### Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

### Technical Note:

This antibody has been optimized for use with Biocare's MACH 4 Detection System. Use TBS buffer for washing steps.

### 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 (NaN<sub>3</sub>) 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>.

### 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.
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 expressions 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.



60 Berry Drive

Pacheco, CA 94553

USA

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Tel: 800-799-9499 | [www.biocare.net](http://www.biocare.net) | Fax: 925-603-8080