

MSH6

Concentrated and Prediluted Monoclonal Antibody
901-265-121021

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M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 265 AK, BK, CK	0.1, 0.5, 1.0 mL	1:100	Renoir Red
Predilute	PM 265 AA, H	6.0, 25 mL	Ready-to-use	N/A
intelliPATH FLX	IPI 265 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 265 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 265 T60	60 tests	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 265 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 265 G7	7.0 mL	Ready-to-use	N/A

Intended Use:

For In Vitro Diagnostic Use

MSH6 [44] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of MSH6 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:

MSH6 is a heterodimer of MSH2 and binds to DNA containing G/T mismatches. The MSH2-MSH6 complex recognizes a single-based mispair insertion/deletion loop. 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) (1,2). These defects in DNA repair pathways have been related to human carcinogenesis. Studies have shown the mutations in MSH-1, MSH2, MSH6, and PMS2 genes contribute to the development of sporadic colorectal carcinoma. The repair of mismatch DNA is essential to maintaining the integrity of genetic information over time (1,2). Recently, a heterogeneous pattern of MSH6 expression was described in rare cases of colorectal, endometrial and sebaceous tumors (2). In this pattern, areas of strong MSH6 expression are juxtaposed with areas of complete loss of expression. 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 (3). PD-L1 IHC test has been demonstrated to be a useful predictive marker for anti-PD-1 immunotherapy in colorectal carcinoma (4).

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: 44 (previously known as BC/44)

Isotype: IgG1

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: MSH6

Cellular Localization: Nuclear

Positive Tissue Control: Colon cancer

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative
Renoir Red Diluent (PD904)

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:

IPI265 is intended for use on 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):

OAI265 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: MSH6

Protocol Template (Description): Ms HRP Template 1

Dewaxing (DS Option): DS2

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

Reagent Name, Time, Temp.: MSH6, 30 min., 25°C

Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI265 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: MSH6

Protocol Template (Description): Ms HRP Template 1

Dewaxing (DS Buffer Option): DS2-50

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

Block Option: Buffer

Reagent Name, Time, Temp.: MSH6, 45 min., 25°C

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Protocol Recommendations (Ventana BenchMark ULTRA):

AVI265 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: CC1 48 minutes

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: 24 minutes, 37°C

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

ALI265 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

Detection: Bond Polymer Refine

HIER: 20 min with ER2

Peroxide Block: 5 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) (5)

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. (6)

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. Renkonen E, *et al.* Altered expression of MLH1, MSH2, and MSH6 in predisposition to hereditary nonpolyposis colorectal cancer. *J Clin Oncol.* 2003;21:3629-37.
2. Graham RP, *et al.* Heterogenous MSH6 loss is a result of microsatellite instability within MSH6 and occurs in sporadic and hereditary colorectal and endometrial carcinomas. *Am J Surg Pathol.* 2015;39:1370-6.
3. Lee LH, *et al.* Patterns and prognostic relevance of PD-1 and PD-L1 expression in colorectal carcinoma. *Mod Pathol.* 2016;29:1333-42.
4. Le DI, *et al.* PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med.* 2015;372:2509-20.
5. 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."
6. 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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