FLEX
Monoclonal Mouse
Anti-Human MSH2
Clone FE11
Ready-to-Use
(Dako Omnis)

Code GA085

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human MSH2, Clone FE11, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry (IHC) together with the Dako Omnis instrument. This antibody labels MSH2-expressing cells in formalin-fixed, paraffin-embedded (FFPE) tissue. Results aid in the classification of colorectal cancer (CRC). Differential classification is aided by the results from a panel of antibodies. 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. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Summary and explanation

Antibodies to MSH2 are useful for identifying MSH2 (mutS protein homolog 2) protein in tumors of the gastrointestinal tract and associated extracolonic cancers by IHC. The absence of MSH2 expression as determined by IHC has been reported in colon carcinoma associated with hereditary nonpolyposis colorectal cancer (1).

Refer to our *General Instructions for Immunohistochemical Staining* and the visualization system instructions of IHC procedures for: Principle of Procedure; Materials Required, Not Supplied; Storage; Specimen Preparation; Staining Procedure; Quality Control; Troubleshooting; Interpretation of Staining; General Limitations.

Reagent provided

Ready-to-use monoclonal antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.

Clone: FE11. Isotype: IgG1, kappa.

Reagent required, but not provided

Refer to our *General Instructions for Immunohistochemical Staining* and the visualization system instructions of IHC procedures.

EnVision FLEX, High pH (Dako Omnis) (Code GV800 or GV823)
EnVision FLEX+ Mouse LINKER (Dako Omnis) (Code GV821)
EnVision FLEX+ Rabbit LINKER (Dako Omnis) (Code GV809)
Wash Buffer (20x) (Dako Omnis) (Code GC807)
Dako Omnis Sulfuric Acid, 0.3 M (Code GC203)
Hematoxylin (Dako Omnis) (Code GC808) or equivalent
Clearify Clearing Agent (Code GC810)
Distilled or de-ionized water (Reagent-grade quality water)*
Drying oven, capable of maintaining 60 °C or less
Ethanol, absolute and 95%
Xylene, or xylene substitute
Bright field microscope (4–20x objective magnification)
Coverslips
Permanent mounting medium and ancillary reagents required for mounting coverslips
Microscope slides: FLEX IHC Microscope Slides (Code K8020) or SuperFrost Plus slides
Tissues to use as process controls (see Quality control section)

*Note: Not all sources of distilled or de-ionized water may be of sufficient quality for IHC reagent preparation. Agilent recommends reagent-grade distilled or de-ionized water [corresponding to Clinical Laboratory Reagent Water (CLRW) standard as specified by CLSI (2)], or water similar in quality to be used for reagent preparation.

Immunogen

Recombinant protein comprising the carboxyl-terminal 330 amino acids of hMSH2.

Specificity

In Western blotting of human Colo-205 cell lysate, the antibody labels a major band at 105 kDa corresponding to the expected molecular weight of MSH2.

Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. For prescription use only (Rx Only).
4. This product contains sodium azide (NaN3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing (3).
5. As with any product derived from biological sources, proper handling procedures should be used in accordance with local requirements.
6. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
7. Unused solution should be disposed of as chemical/biological waste in accordance with all local, regional, national and international regulations.
8. Incubation times, temperatures, or methods other than those specified may give erroneous results.
9. Reagent has been optimally diluted. Further dilution may result in loss of visible immunoreactivity.
10. Contact Agilent Pathology Support via www.agilent.com to report any unusual staining. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the country in which the user and/or the patient is established.
11. Safety Data Sheets are available on www.agilent.com or on request.

Storage

Store at 2–8 °C. During storage the cap should be closed. Do not use after expiration date stamped on vial. Onboard stability is 375 hours. Onboard time is tracked by the Dako Omnis Software. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Agilent Pathology Support.

Staining protocol overview*

Step	**Reagent**	**Protocol**
Deparaffinization	Clearify (Code GC810)	Default
Pre-treatment	EnVision FLEX, High pH (Code GV800 or GV823 or GV804)	30 min heat-induced epitope retrieval
Primary antibody	Ready-to-Use (Code GA085)	20 min incubation
Negative control reagent	FLEX Negative Control, Mouse (Code GA750)	25 min incubation
Visualization	EnVision FLEX (Code GV800) + EnVision FLEX+ Mouse LINKER (Code GV821) + EnVision FLEX+ Rabbit LINKER (Code GV809)	Block: 3 min; Mouse Link: 10 min; Rabbit Link: 10 min; Polymer: 20 min; Chromogen: 5 min
Counterstain	Hematoxylin (Code GC808)	3 min incubation
Mounting	Nonaqueous, permanent mounting required	Dehydration, clearing and mounting must be performed after unloading
Slides	FLEX IHC Microscope Slides (Code K8020) or Superfrost Plus slides	Recommended for greater adherence of tissue sections to glass slides
Quality control	**Tissue**	**Staining pattern**
Control tissue	Intestinal Tract	Nuclear

**The user must always read the package inserts for the reagents used and consult the Dako Omnis User Guides for details.*

Specimen preparation

Paraffin-embedded tissue:
FFPE tissues are suitable for use. Alternative fixatives have not been validated and may give erroneous results. Fixation time for 6–48 hours in 10% neutral buffered formalin (NBF) is recommended. Specimens should be blocked into a thickness of 3 or 4 mm, fixed in formalin and dehydrated and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. Fixation times outside of 6–48 hours should be validated by the user.

Paraffin sections: The antibody can be used for labeling FFPE tissue sections. Tissue specimens should be cut into sections of approximately 4 µm. After sectioning, tissues should be mounted on FLEX IHC Microscope Slides (Code K8020) or SuperFrost Plus microscope slides and then placed in a 58 ± 2 °C calibrated oven for 1 hour.

For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides (Code K8020) is recommended.

To preserve antigenicity, tissue sections mounted on slides should be stained within 6 months of sectioning when held in the dark at 2–8 °C (preferred), or at room temperature up to 25 °C.

Staining procedure

Deparaffinization, target retrieval, immunohistochemical staining and counterstaining are performed onboard Dako Omnis. The staining steps and incubation times are preprogrammed into the Dako Omnis software. The “MSH2 IHC GA085” protocol is used with the primary antibody MSH2. If the protocol is not available in the Dako Omnis system, it can be downloaded from *Dako Omnis Protocol Update* at www.agilent.com or contact Agilent Pathology Support. Please refer to the Dako Omnis Basic User Guide for detailed instructions on loading slides and reagents.

Pre-treatment: Deparaffinization of FFPE tissue sections is performed using Clearify (Code GC810). Target retrieval with heat-induced epitope retrieval (HIER) using diluted EnVision FLEX Target Retrieval Solution, High pH (50x) (Dako Omnis) (Code GV804) is recommended.

Visualization: The recommended visualization system is EnVision FLEX, High pH (Dako Omnis) (Code GV800) in combination with EnVision FLEX+ Mouse (LINKER) (Dako Omnis) (Code GV821) and EnVision FLEX+ Rabbit (LINKER) (Dako Omnis) (Code GV809).

Counterstaining: The recommended counterstain is Hematoxylin (Dako Omnis) (Code GC808).

Mounting: After staining onboard Dako Omnis the sections must be dehydrated, cleared and mounted using a permanent mounting method. Do not allow sections to dry prior to mounting.

Dako Omnis ensures that the tissue sections do not dry out during the pre-treatment process or during the following immunohistochemical staining procedure.

Quality control

Monoclonal Mouse Anti-Human MSH2, Clone FE11 has been quality-control tested by immunohistochemistry using the required reagents and staining procedures outlined above. Deviations in the recommended procedures for tissue fixation, processing and embedding in the user's laboratory may produce significant variability in results.

System level controls
Positive and negative control tissues (lab-supplied) should be run for each staining procedure. These quality controls are intended to ensure the validity of the staining procedure, including reagents, tissue processing and instrument performance. It is recommended that control tissues be stained on the same slide as the patient tissue. The positive control should be a tissue with positive biomarker expression fixed in the same way as the patient tissue. Patient CRC tissues contain positive nonmalignant elements that serve as an internal positive control. Positive nuclear staining of benign or normal epithelium, lymphocytes, and stromal cells should demonstrate moderate to strong staining intensity. The negative control should be a tissue with loss of biomarker expression fixed in the same way as patient tissue. Negative control tissues should exhibit no or equivocal nuclear staining in viable malignant cells compared to moderate or strong nuclear staining in adjacent internal positive controls. If controls are not fixed in the same way as the patient tissue, the control may only be used as a staining control for reagents and instrument performance.

Negative control reagent
Negative control reagent (NCR) may be used in place of the primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. The recommended negative control reagent is FLEX Negative Control, Mouse (Dako Omnis) (Code GA750).

Staining interpretation

The cellular staining pattern is nuclear.

Performance characteristics

Normal tissues:

Tissue Type (# tested)	Labeled Tissue Elements	Tissue Type (# tested)	Labeled Tissue Elements	Tissue Type (# tested)	Labeled Tissue Elements
Adrenal (3)	3/3	Lung (3)	3/3	Salivary gland (3)	3/3
Bladder (3)	3/3	Mesothelial cells (3)	3/3	Skin (3)	3/3
Bone marrow (3)	3/3*	Muscle, cardiac (3)	3/3*	Small intestine (3)	3/3
Breast (3)	2/3	Muscle, skeletal (3)	3/3	Spleen (3)	3/3
Cerebellum (3)	3/3	Nerve, peripheral (3)	3/3	Stomach (3)	3/3
Cerebrum (3)	1/3	Ovary (3)	3/3	Testis (3)	3/3
Cervix (3)	3/3	Pancreas (3)	3/3	Thymus (3)	3/3**
Colon (3)	3/3	Parathyroid (3)	3/3	Thyroid (3)	3/3
Esophagus (3)	3/3	Pituitary (3)	3/3	Tonsil (3)	3/3
Kidney (3)	3/3	Prostate (3)	3/3	Uterus (3)	3/3
Liver (3)	3/3*				

*cytoplasmic staining pattern for at least one case

**cytoplasmic and extracellular staining pattern for at least one case

Abnormal tissues:

Tissue Type (# tested)	Labeled Tissue Elements	Tissue Type (# tested)	Labeled Tissue Elements
Bladder carcinoma (2)	2/2	Ovarian granulosa cell tumor (1)	1/1
Breast carcinoma (5)	5/5	Islet cell tumor of pancreas (1)	1/1
Cholangiocarcinoma (1)	1/1	Pancreatic glucagonoma (1)	1/1
Colon adenocarcinoma (1)	1/1	Pleomorphic rhabdomyosarcoma (1)	1/1
Endometrial sarcoma (1)	1/1	PNET scrotum (1)	1/1
Ewing's sarcoma (1)	1/1	Prostate adenocarcinoma (2)	2/2
Gastric adenocarcinoma (2)	2/2	Prostate benign prostatic hyperplasia (1)	1/1
Hepatoma (1)	1/1	Renal cell carcinoma (1)	1/1

Kidney transitional cell carcinoma (1)	1/1	Squamous carcinoma of ear (1)	1/1
Liver cell adenoma (1)	1/1	Testicular embryonal carcinoma (1)	1/1
Lung carcinoma (4)	4/4	Testicular yolk sac tumor (1)	1/1
Lymphoma of cecum (1)	1/1	Thymic carcinoid tumor (1)	1/1
Melanoma (3)	3/3	Thymoma (1)	1/1
Merkel cell tumor (1)	1/1	Thyroid carcinoma (2)	2/2
Ovarian carcinoma (2)	2/2	Uterine adenomatoid tumor (1)	1/1
Ovarian dysgerminoma (1)	1/1		

Precision in CRC tissues:

Precision Study	**Study Design**	**% Agreement (95% Confidence Interval)**
Intra-rack (Repeatability)	24 CRC specimens (12 MSH2 Intact and 12 MSH2 Loss) were tested with 4 replicates within a rack on the Dako Omnis instrument.	NPA 100% (92.6–100%) PPA 100% (92.6–100%) OA 100% (96.2–100%)
Inter-Rack	24 CRC specimens (12 MSH2 Intact and 12 MSH2 Loss) were tested with a single replicate on 4 different racks within the same Dako Omnis instrument.	NPA 100% (92.6–100%) PPA 100% (92.6–100%) OA 100% (96.2–100%)
Inter-Instrument	24 CRC specimens (12 MSH2 Intact and 12 MSH2 Loss) were tested with 2 replicates on each of 3 Dako Omnis instruments.	NPA 100% (94.9–100%) PPA 100% (94.9–100%) OA 100% (97.4–100%)
Inter-Day	24 CRC specimens (12 MSH2 Intact and 12 MSH2 Loss) were tested with a single replicate on the Dako Omnis instrument over 5 nonconsecutive days.	NPA 100% (94.0–100%) PPA 100% (94.0–100%) OA 100% (96.9–100%)
Inter-Lot	24 CRC specimens (12 MSH2 Intact and 12 MSH2 Loss) were tested with 2 replicates on the Dako Omnis instrument with 3 lots of reagent.	NPA 98.6% (95.8–100%) PPA 98.6% (95.8–100%) OA 98.6% (96.5–100%)

NPA=Negative Percent Agreement; PPA=Positive Percent Agreement; OA=Overall Percent Agreement

References

1. Lanza G, Gafá R, Maestri I, Santini A, Matteuzzi M, Cavazzini L. Immunohistochemical pattern of MLH1/MSH2 expression is related to clinical and pathological features in colorectal adenocarcinomas with microsatellite instability. Mod Pathol 2002; 15:741-49.
2. CLSI. Preparation and testing of reagent water in the clinical laboratory: Approved guideline – fourth edition. CLSI document GP40-A4-AMD. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
3. Department of Health, Education and Welfare, National Institutes for Occupational Safety and Health, Rockville, MD. “Procedures for the decontamination of plumbing systems containing copper and/or lead azides.” DHHS (NIOSH) Publ. No. 78-127, Current 13. August 16, 1976.

Explanation of symbols

REF	Catalogue number	2 °C 8 °C	Temperature limitation		Use by
IVD	In vitro diagnostic medical device	LOT	Batch code		Manufacturer
	Consult instructions for use	EC REP	Authorized representative in the European Community		

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