CONFIRM anti-MSH6 (44) Mouse Monoclonal Primary Antibody

REF 790-4455

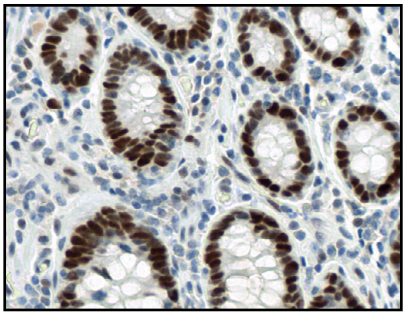

Figure 1. CONFIRM anti-MSH6 (44) Mouse Monoclonal Primary Antibody staining of colon carcinoma

INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-MSH6 (44) Mouse Monoclonal Antibody is used to qualitatively identify human DNA mismatch repair (MMR) protein MSH6, expressed in the nucleus of normal proliferating cells. Deficient or low levels of MSH6 are associated with colorectal and other cancers. The antibody is intended for qualitative staining in sections of formalin fixed, paraffin embedded tissue.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information and proper controls.

This antibody is intended for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

CONFIRM anti-MSH6 (44) Mouse Monoclonal Antibody recognizes MSH6, which is one of several clinically important mismatch repair (MMR) proteins that are mutated in families with hereditary non-polyposis colorectal cancer (HNPCC).[1,2,3] Carriers of these mutations have a high lifetime risk of developing colorectal and several other cancers due to accumulation of DNA replication errors in proliferating cells, a phenomenon known as microsatellite instability (MSI). In normal cells, the MSH6 protein forms complexes (heterodimers) with MSH2 protein.[4,5] These heterodimers induce conformational changes in the DNA that lead to binding of another heterodimer complex of MSH1 PMS proteins and excision repair of the affected DNA. Mutations or deficiencies in these proteins result in frequent MSI and somatic mutation due to replication error.

HNPCC represents 1-3% of all colorectal cancers, with MSH6 loss occurring in 9% of these (nearly 90% of HNPCC cases show loss of MSH1 or MSH2).[6] More than 300 different mutations in the MMR family of proteins have been identified in people with HNPCC. The HNPPC-associated tumor phenotype is generally characterized by immunohistochemical loss of expression in MMR proteins, particularly MLH1, MSH2, MSH6 and PMS2.[7,8] Deficiencies in these proteins is closely related to the degree of MSI in a tumor. MMR protein deficiencies are thus used in classification of tumors as MSI-H (high degree of MSI), MSI-L (low degree of MSI) or MSS (MS stable).[9] Each classification has implications in treatment and prognosis of the tumor. Patients classified as MSI-H, despite having a much higher likelihood of developing cancer, also have a significant survival advantage, independent of stage or grade, over patients with MSS, which is typically characterized by chromosomal instability. A distinguishing feature of MSH6-associated HPNCC is the observation that many carriers exhibit the MSI-L or MSS tumor phenotype. Functional redundancy in the DNA mismatch repair system could explain the less extensive MSI observed in tumors with the MSH6 germline mutation.[4] Persons found to be deficient in MSH2 are often also deficient in MSH6, possibly due to protein instability when heterodimerization cannot occur.

REAGENT PROVIDED

CONFIRM anti-MSH6 (44) Mouse Monoclonal Primary Antibody contains sufficient reagent for staining 50 tests.

One 5 mL dispenser of CONFIRM anti-MSH6 (44) contains approximately 0.505 µg of a mouse monoclonal antibody.

The antibody is diluted in a phosphate buffered saline containing carrier protein and a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 0.101 µg/mL. There is no known non specific antibody reactivity observed in this product.

CONFIRM anti-MSH6 (44) is a mouse monoclonal antibody harvested from tissue cell supernatant.

This antibody is optimized for use on a Ventana automated slide stainer in combination with Ventana detection kits. No reconstitution, mixing, dilution, or titration is required.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of: (1) Principles and Procedures, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (ie, *ultra*View Universal DAB Detection Kit), and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, after every use the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin fixed, paraffin embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[10] Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents.
5. Consult local and/or state authorities with regard to recommended method of disposal.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark XT and BenchMark ULTRA instruments with *ultra*View Universal DAB Detection Kit is listed in Table 1.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate Ventana detection package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocols for CONFIRM anti-MSH6 (44) with *ultra*View Universal DAB Detection Kit on BenchMark XT/BenchMark ULTRA instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 16 minutes, 37°C BenchMark ULTRA instrument Approximately 20 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[11]

POSITIVE TISSUE CONTROL

Examples of positive staining cells for CONFIRM anti-MSH6 (44) are expressed in colon carcinoma and breast carcinoma.

STAINING INTERPRETATION

The cellular staining pattern for CONFIRM anti-MSH6 (44) is nuclear.

SPECIFIC LIMITATIONS

CONFIRM anti-MSH6 (44) has been optimized on Ventana BenchMark XT and BenchMark ULTRA instruments in combination with *ultra*View Universal DAB Detection Kit (REF 760-500) at a 16 minute or 20 minute primary antibody incubation time respectively. However, the user must validate individual laboratory results obtained with this reagent. Positive staining of proliferating cells in both normal and neoplastic tissues was observed with CONFIRM anti-MSH6 (44).

PERFORMANCE CHARACTERISTICS

1. Specificity of CONFIRM anti-MSH6 (44) Mouse Monoclonal Primary Antibody was determined by testing formalin fixed paraffin embedded normal and neoplastic tissues.

   For normal tissues, results are as follows: (0/3) cerebrum, (0/3) cerebellum, (3/3) adrenal gland, (0/3) ovary, (2/3) pancreas, (2/2) parathyroid gland, (0/3) hypophysis, (3/3) testis, (1/3) thyroid gland, (3/3) breast, (1/3) spleen, (2/3) tonsil, (2/3) thymus, (0/3) myeloid, (0/3) pulmonary, (0/3) myocardium, (1/3) esophagus, (1/3) gastric fundus, (2/3) small intestine, (3/3) colon, (0/3) liver, (1/3) salivary gland, (1/2) kidney, (2/3) prostate, (1/3) endometrium, (2/3) cervix, (0/3) skeletal muscle, (3/3) skin, (0/3) nerve, (1/3) mesothelium and lung.

   Neoplastic tissues stained included: (1/1) atypical meningioma, (1/1) malignant ependymoma, (0/1) malignant oligodendroglioma, (1/1) ovarian serous papillary adenocarcinoma, (1/1) ovarian mucinous papillary adenocarcinoma, (1/1) islet cell carcinoma, (1/1) pancreatic adenocarcinoma, (1/1) seminoma, (1/1) embryonal carcinoma, (1/1) medullary carcinoma, (1/1) thyroid papillary carcinoma, (1/1) intraductal carcinoma, (2/2) breast carcinoma, (1/1) diffuse B cell lymphoma, (1/1) lung small cell undifferentiated carcinoma, (1/1) lung squamous cell carcinoma, (0/1) lung adenocarcinoma, (1/1) esophageal squamous cell carcinoma, (1/1) esophageal adenocarcinoma, (1/1) colon adenocarcinoma, (1/1) gastric adenocarcinoma, (1/1) intestinal adenocarcinoma, (0/1) intestinal interstitialoma, (0/1) colon interstitialoma, (1/1) rectal adenocarcinoma, (1/1) rectal interstitialoma, (0/1) hepatocellular carcinoma, (0/1) hepatoblastoma, (1/1) clear cell carcinoma, (0/1) prostatic adenocarcinoma, (1/1) prostatic transitional cell carcinoma, (0/1) leiomyoma, (0/1) endometrial adenocarcinoma, (0/1) endometrial clear cell carcinoma, (1/3) squamous cell carcinoma, (1/1) embryonal rhabdomyosarcoma, (0/1) malignant melanoma, (0/1) basal cell carcinoma, (0/1) neurofibroma, (1/1) neuroblastoma, (1/1) epithelial malignant mesothelioma, (2/2) diffuse malignant lymphoma, (1/1) Hodgkin's lymphoma, (1/1) diffuse malignant lymphoma, (1/1) bladder transitional cell carcinoma with squamous cell metaplasia, (1/2) leiomyosarcoma, (1/1) femur osteosarcoma, (0/1) spindle cell rhabdomyosarcoma, (0/1) malignant melanoma, (47/79) adenocarcinoma, (12/19) mucinous adenocarcinoma, (2/2) tubulo-villous adenocarcinoma, (1/4) tubular adenocarcinoma, (2/2) adenosquamous carcinoma, (2/2) neuroendocrine carcinoma, (2/2) leiomyosarcoma, (10/10) normal colon, (2/4) smooth muscle of colon, (8/9) cancer adjacent lymph node metastatic adenocarcinoma, (22/30) cancer adjacent colon tissue, (3/4) cancer adjacent rectum tissue, and (2/9) lymph node tissue.
2. Lot to lot reproducibility was determined by testing 3 lots across 1 multi-tissue block (3 tissues per block) on a BenchMark XT instrument. 3 out of 3 samples across all 3 lots scored equivalently.
3. Inter-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 5 slides on a BenchMark XT instrument over a five day non-consecutive period. 149 out of 150 samples tested scored equivalently.
4. Intra-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 14 slides on a BenchMark XT instrument. 83 out of 84 samples tested scored equivalently.
5. Intra-platform repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block) across 5 slides on 3 BenchMark XT instruments. 90 out of 90 samples tested scored equivalently.
6. Inter-platform repeatability was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark XT instruments and 3 BenchMark ULTRA instruments. 90 out of 90 samples tested scored equivalently.
7. Compatible with *i*VIEW DAB and *ultra*View Universal DAB Detection Kits.

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