abcam

Product datasheet

Anti-MSH2 antibody [EPR21017-123] ab227941

KO VALIDATED Recombinant RabMAb

10 References 10 Images

Overview

Product name	Anti-MSH2 antibody [EPR21017-123]
Description	Rabbit monoclonal [EPR21017-123] to MSH2
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, A549 and A-375 whole cell lysates; Human tonsil and fetal heart lysates. IHC-P: Human testis and colon cancer tissues. ICC/IF: A-375 and A549 cells. Flow Cyt (intra): A-375 cells. HAP1-WT cells. IP: A-375 whole cell lysate.
General notes	To see more of the key markers and tools you need to study the hallmarks of cancer, including genome instability and mutation, please visit the following **page**. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR21017-123
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227941 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use a concentration of 1 µg/ml. MeOH fixationis recommended
WB		1/1000. Detects a band of approximately 104 kDa (predicted molecular weight: 104 kDa).
ICC/IF		1/100.
IP		1/30.
IHC-P		1/8000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function Component of the post-replicative DNA mismatch repair system (MMR). Forms two different heterodimers: MutS alpha (MSH2-MSH6 heterodimer) and MutS beta (MSH2-MSH3 heterodimer) which binds to DNA mismatches thereby initiating DNA repair. When bound, heterodimers bend the DNA helix and shields approximately 20 base pairs. MutS alpha recognizes single base mismatches and dinucleotide insertion-deletion loops (IDL) in the DNA. MutS beta recognizes larger insertion-deletion loops up to 13 nucleotides long. After mismatch binding, MutS alpha or beta forms a ternary complex with the MutL alpha heterodimer, which is thought to be responsible for directing the downstream MMR events, including strand discrimination, excision, and resynthesis. ATP binding and hydrolysis play a pivotal role in mismatch repair functions. The ATPase activity associated with MutS alpha regulates binding similar to a molecular switch: mismatched DNA provokes ADP-->ATP exchange, resulting in a discernible conformational transition that converts MutS alpha into a sliding clamp capable of hydrolysis-independent diffusion along the DNA backbone. This transition is crucial for mismatch repair. MutS alpha may also play a role in DNA homologous recombination repair. In melanocytes may modulate both UV-B-induced cell cycle regulation and apoptosis.

Tissue specificity Ubiquitously expressed.

Involvement in disease Defects in MSH2 are the cause of hereditary non-polyposis colorectal cancer type 1 (HNPCC1) [MIM:120435]. Mutations in more than one gene locus can be involved alone or in combination in the production of the HNPCC phenotype (also called Lynch syndrome). Most families with clinically recognized HNPCC have mutations in either MLH1 or MSH2 genes. HNPCC is an autosomal, dominantly inherited disease associated with marked increase in cancer susceptibility. It is characterized by a familial predisposition to early onset colorectal carcinoma (CRC) and extra-colonic cancers of the gastrointestinal, urological and female reproductive tracts. HNPCC is reported to be the most common form of inherited colorectal cancer in the Western

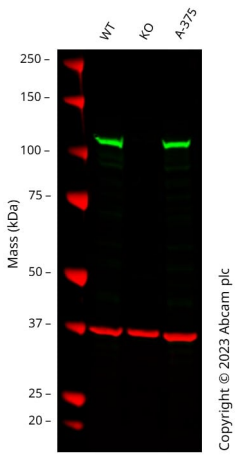
world. Cancers in HNPCC originate within benign neoplastic polyps termed adenomas. Clinically, HNPCC is often divided into two subgroups. Type I: hereditary predisposition to colorectal cancer, a young age of onset, and carcinoma observed in the proximal colon. Type II: patients have an increased risk for cancers in certain tissues such as the uterus, ovary, breast, stomach, small intestine, skin, and larynx in addition to the colon. Diagnosis of classical HNPCC is based on the Amsterdam criteria: 3 or more relatives affected by colorectal cancer, one a first degree relative of the other two; 2 or more generation affected; 1 or more colorectal cancers presenting before 50 years of age; exclusion of hereditary polyposis syndromes. The term "suspected HNPCC" or "incomplete HNPCC" can be used to describe families who do not or only partially fulfill the Amsterdam criteria, but in whom a genetic basis for colon cancer is strongly suspected. MSH2 mutations may predispose to hematological malignancies and multiple cafe-au-lait spots. Defects in MSH2 are a cause of Muir-Torre syndrome (MuToS) [MIM:158320]; also abbreviated MTS. MuToS is a rare autosomal dominant disorder characterized by sebaceous neoplasms and visceral malignancy.
Defects in MSH2 are a cause of susceptibility to endometrial cancer (ENDMC) [MIM:608089].
Defects in MSH2 are a cause of hereditary non-polyposis colorectal cancer type 8 (HNPCC8) [MIM:613244]. HNPCC is a disease associated with marked increase in cancer susceptibility. It is characterized by a familial predisposition to early-onset colorectal carcinoma (CRC) and extra-colonic tumors of the gastrointestinal, urological and female reproductive tracts. HNPCC is reported to be the most common form of inherited colorectal cancer in the Western world. Clinically, HNPCC is often divided into two subgroups. Type I is characterized by hereditary predisposition to colorectal cancer, a young age of onset, and carcinoma observed in the proximal colon. Type II is characterized by increased risk for cancers in certain tissues such as the uterus, ovary, breast, stomach, small intestine, skin, and larynx in addition to the colon. Diagnosis of classical HNPCC is based on the Amsterdam criteria: 3 or more relatives affected by colorectal cancer, one a first degree relative of the other two; 2 or more generation affected; 1 or more colorectal cancers presenting before 50 years of age; exclusion of hereditary polyposis syndromes. The term 'suspected HNPCC' or 'incomplete HNPCC' can be used to describe families who do not or only partially fulfill the Amsterdam criteria, but in whom a genetic basis for colon cancer is strongly suspected. Note=HNPCC8 results from heterozygous deletion of 3-prime exons of EPCAM and intergenic regions directly upstream of MSH2, resulting in transcriptional read-through and epigenetic silencing of MSH2 in tissues expressing EPCAM.

Sequence similarities Belongs to the DNA mismatch repair mutS family.

Post-translational modifications Phosphorylated by PRKCZ, which may prevent MutS alpha degradation by the ubiquitin-proteasome pathway.
Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization Nucleus.

Images



Western blot - Anti-MSH2 antibody [EPR21017-123] (ab227941)

All lanes : Anti-MSH2 antibody [EPR21017-123] (ab227941) at 1/1000 dilution

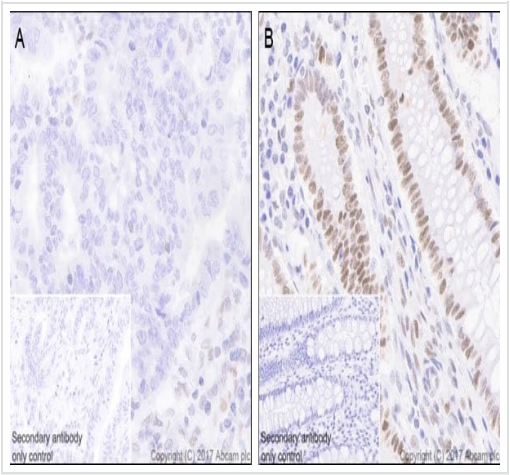
Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : MSH2 knockout HCT 116 cell lysate
Lane 3 : A-375 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 104 kDa
Observed band size: 104 kDa

Western blot: Anti-MSH2 antibody [EPR21017-123] (ab227941) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab227941 was shown to bind specifically to MSH2. A band was observed at 104 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in MSH2 knockout cell line. To generate this image, wild-type and MSH2 knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

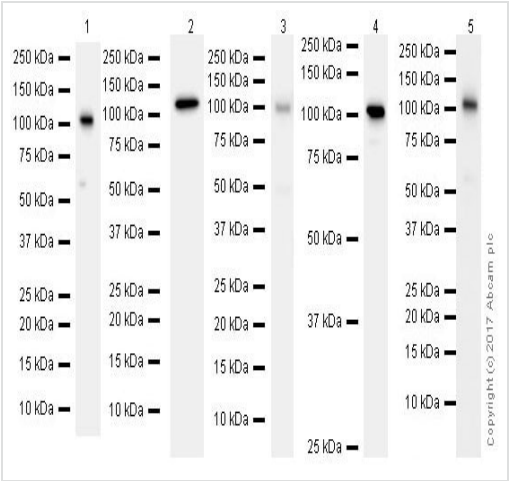


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH2 antibody [EPR21017-123] (ab227941)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling MSH2 with ab227941 at 1/8000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in para-carcinoma colonic epithelium (image B) or stromal cells (both image A and B) and loss of expression in the paired colon cancer (image A) (PMID: 24710284). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-MSH2 antibody [EPR21017-123] (ab227941)

All lanes : Anti-MSH2 antibody [EPR21017-123] (ab227941) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : A549 (human lung carcinoma epithelial cell), whole cell lysate
Lane 3 : Human fetal heart lysate
Lane 4 : Human tonsil lysate
Lane 5 : A-375 (human malignant melanoma epithelial cell), whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

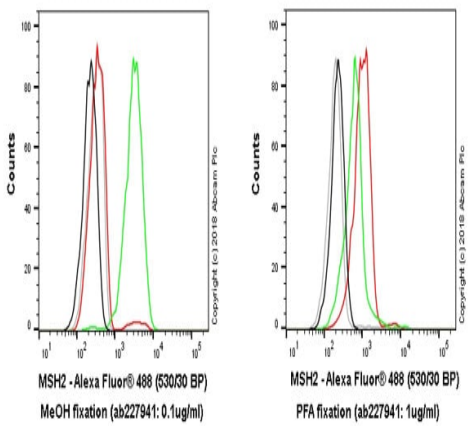
Developed using the ECL technique.

Predicted band size: 104 kDa
Observed band size: 104 kDa

Exposure times: Lane 1: 100 seconds; Lane 2: 3 minutes; Lane 3: 10 seconds; Lane 4: 1 minute; Lane 5: 15 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument.



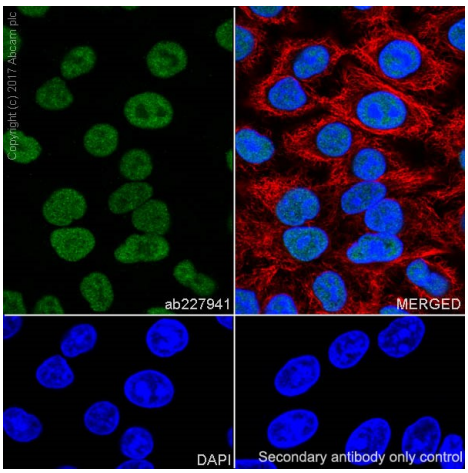
Flow Cytometry (Intracellular) - Anti-MSH2 antibody [EPR21017-123] (ab227941)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-MSH2 knockout cells (red line) stained with ab227941. The cells were fixed with 80% methanol (5 min) (left panel) or 4% formaldehyde (10 min) (right panel) , and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by ab227941 for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C.

A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-MSH2 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

Note: We recommend fixing cells using MeOHinstead of PFA toget optimal results.

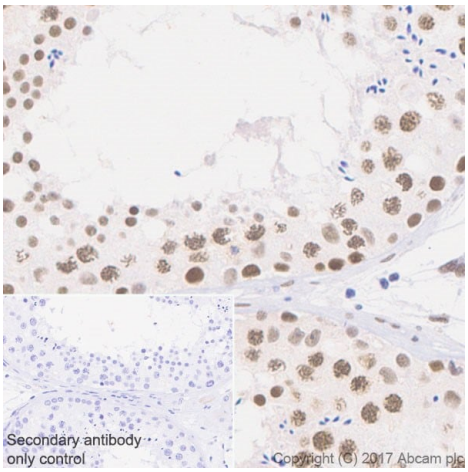


Immunocytochemistry/ Immunofluorescence - Anti-MSH2 antibody [EPR21017-123] (ab227941)

Immunofluorescent analysis of 100% methanol-fixed A549 (human lung carcinoma cell line) cells labeling MSH2 with ab227941 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Nuclear staining in A549 cell line is shown.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

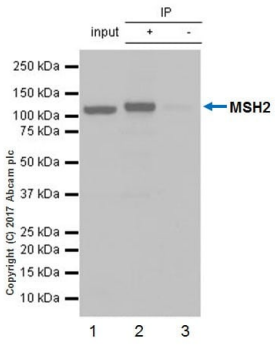


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH2 antibody [EPR21017-123] (ab227941)

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling MSH2 with ab227941 at 1/8000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in human testis was observed (PMID: 10029069). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunoprecipitation - Anti-MSH2 antibody [EPR21017-123] (ab227941)

MSH2 was immunoprecipitated from 0.35 mg of A-375 (human malignant melanoma cell line) lysate with ab227941 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab227941 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

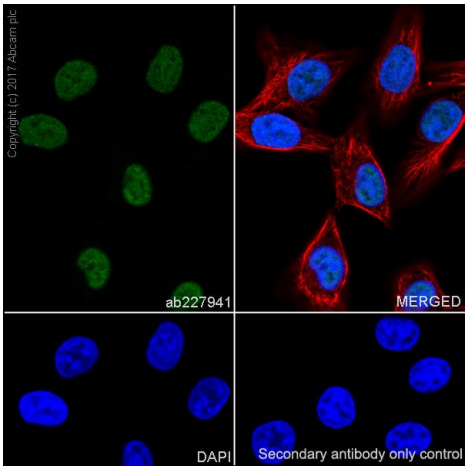
Lane 1: A-375 whole cell lysate 10 µg (Input).

Lane 2: ab227941 IP in A-375 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab227941 in A-375 whole cell lysate.

Exposure time: 1 second.

Blocking and dilution buffer concentration: 5% NFDM/TBST.

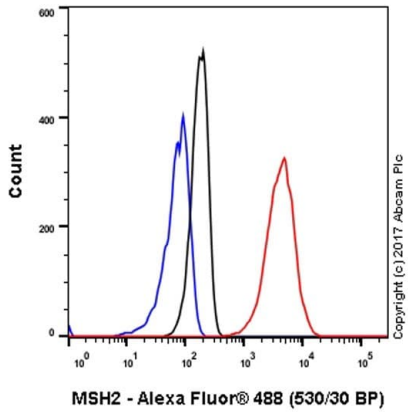


Immunocytochemistry/ Immunofluorescence - Anti-MSH2 antibody [EPR21017-123] (ab227941)

Immunofluorescent analysis of 100% methanol-fixed A-375 (human malignant melanoma cell line) cells labeling MSH2 with ab227941 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Nuclear staining in A-375 cell line is shown.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-MSH2 antibody [EPR21017-123] (ab227941)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized A-375 (humanmalignant melanoma cell line) cell line labeling MSH2 with ab227941 at 1/600 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

Anti-MSH2 antibody [EPR21017-123] (ab227941)

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