

Product datasheet

Anti-MSH6 antibody [SP93] ab99889

KO VALIDATED

Recombinant

RabMAb

[1 References](#) [10 Images](#)

Overview

Product name	Anti-MSH6 antibody [SP93]
Description	Rabbit monoclonal [SP93] to MSH6
Host species	Rabbit
Tested applications	Suitable for: WB, ICC/IF, IHC-P, Flow Cyt (Intra)
Species reactivity	Reacts with: Mouse, Human
Immunogen	Synthetic peptide within Human MSH6 aa 350-450 (internal sequence). The exact sequence is proprietary. Database link: P52701
Epitope	Internal region
Positive control	IHC-P: Human rectal carcinoma, colon, and colon carcinoma tissues; Mouse colon Tissue ICC/IF: HeLa cells, HAP1 cells (HAP1-MSH6 knockout cell line used as negative cell line). Flow Cyt (Intra): HeLa cells.
General notes	<p>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.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C.
Storage buffer	pH: 7.60 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA

Purity	Protein A/G purified
Purification notes	Purified from TCS by protein A/G.
Clonality	Monoclonal
Clone number	SP93
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab99889 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
WB		Use at an assay dependent concentration.
ICC/IF		1/250.
IHC-P		1/100. Antigen retrieval: boil tissue section in 1 mM EDTA, pH 8.0 for 10 minutes followed by cooling at room temperature for 20 minutes. Primary antibody incubation: 30 minutes at room temperature.
Flow Cyt (Intra)		1/20.

Target

Function Component of the post-replicative DNA mismatch repair system (MMR). Heterodimerizes with MSH2 to form MutS alpha, which binds to DNA mismatches thereby initiating DNA repair. When bound, MutS alpha bends the DNA helix and shields approximately 20 base pairs, and recognizes single base mismatches and dinucleotide insertion-deletion loops (IDL) in the DNA. After mismatch binding, 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.

Involvement in disease Defects in MSH6 are the cause of hereditary non-polyposis colorectal cancer type 5 (HNPCC5) [MIM:600678]. 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. MSH6 mutations appear to be associated with atypical HNPCC and in particular with development of endometrial carcinoma or atypical endometrial hyperplasia, the presumed precursor of endometrial cancer. Defects in MSH6 are also found in familial colorectal cancers (suspected or incomplete HNPCC) that do not fulfill the Amsterdam criteria for HNPCC.

Defects in MSH6 are a cause of susceptibility to endometrial cancer (ENDMC) [MIM:608089].

Sequence similarities

Belongs to the DNA mismatch repair mutS family.

Contains 1 PWWP domain.

Post-translational modifications

The N-terminus is blocked.

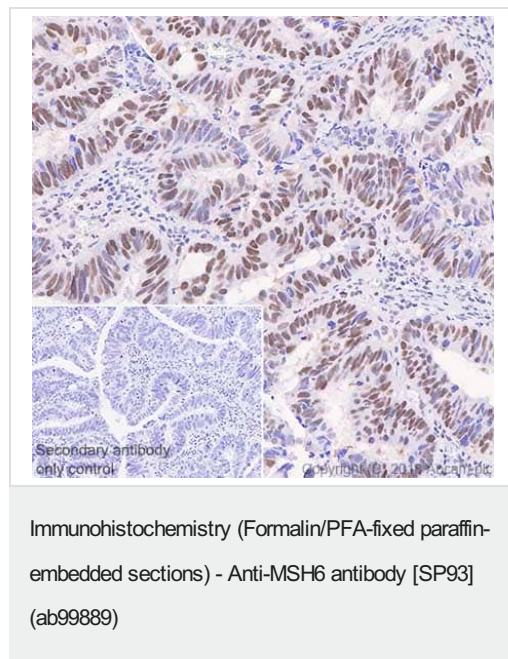
Phosphorylated upon DNA damage, probably by ATM or ATR.

Phosphorylated by PRKCZ, which may prevent MutS alpha degradation by the ubiquitin-proteasome pathway.

Cellular localization

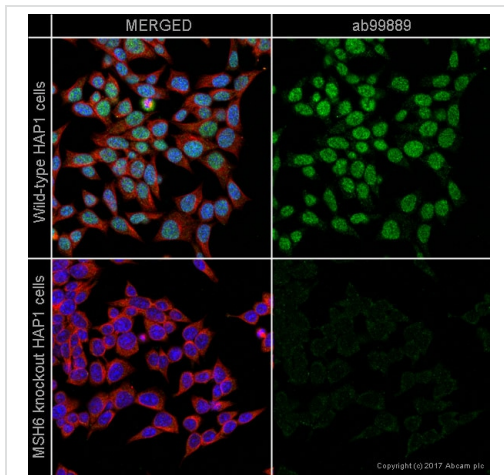
Nucleus.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon carcinoma tissue sections labeling MSH6 with ab99889 at 1/100 dilution (1.0 µg/ml). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)) was used as the secondary antibody. Hematoxylin was used as a counterstain. Nuclear staining on human colon carcinoma, performed on a Leica Biosystems BOND™ RX instrument.

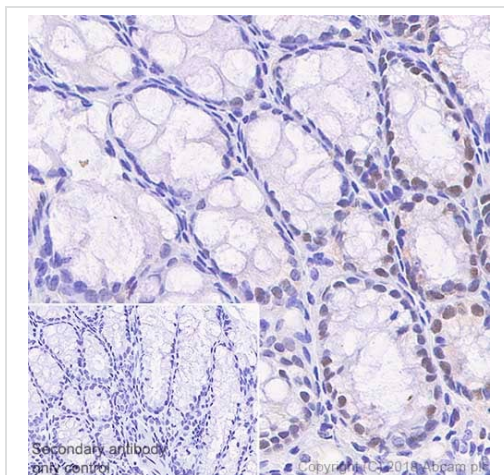
The section was incubated with ab99889 for 30 mins at room temperature.



Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [SP93] (ab99889)

ab99889 staining MSH6 in wild-type HAP1 cells (top panel) and MSH6 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab99889 at 1/250 dilution and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

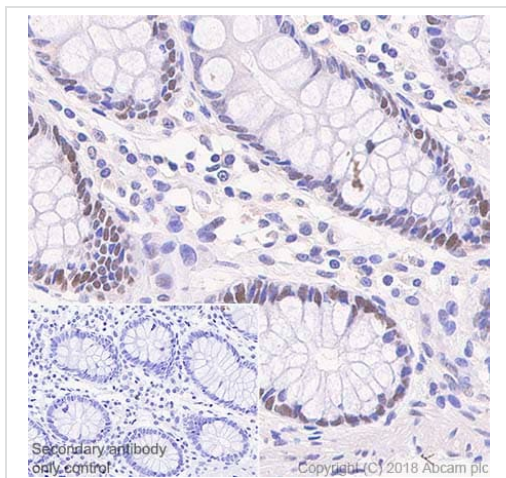
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [SP93] (ab99889)

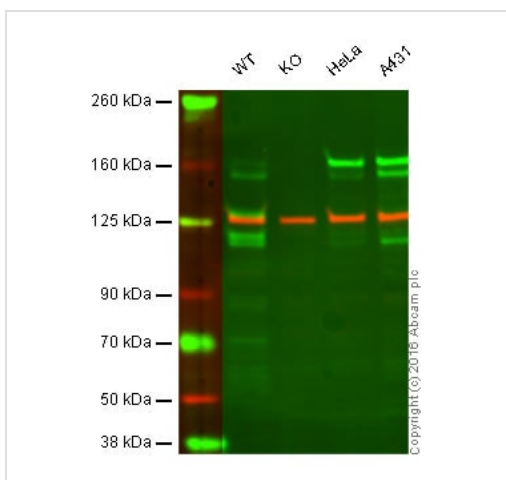
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse colon tissue sections labeling MSH6 with ab99889 at 1/100 dilution (1.0 µg/ml). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Hematoxylin was used as a counterstain. Sporadically nuclear staining on mouse colon, performed on a Leica Biosystems BOND™ RX instrument.

The section was incubated with ab99889 for 30 mins at room temperature.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [SP93] (ab99889)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon tissue sections labeling MSH6 with ab99889 at 1/100 dilution (1.0 µg/ml). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Hematoxylin was used as a counterstain. Sporadically nuclear staining on human colon, performed on a Leica Biosystems BOND™ RX instrument. The section was incubated with ab99889 for 30 mins at room temperature.



Western blot - Anti-MSH6 antibody [SP93] (ab99889)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

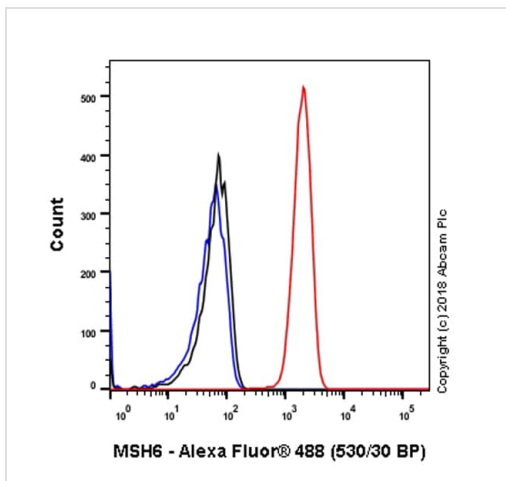
Lane 2: MSH6 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: A431 cell lysate (20 µg)

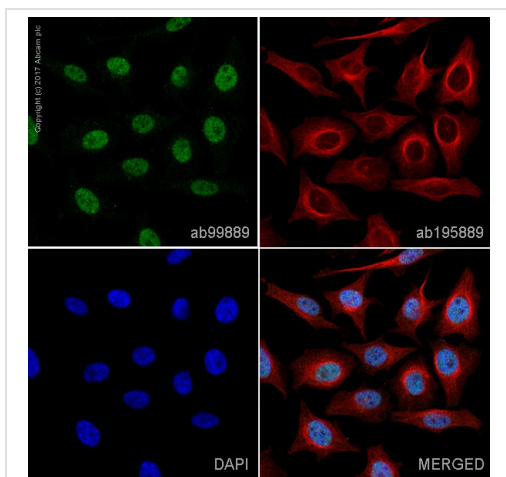
Lanes 1 - 4: Merged signal (red and green). Green - ab99889 observed at 160 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

ab99889 was shown to specifically react with MSH6 in wild-type HAP1 cells along with additional cross reactive bands. No band was observed in MSH6 knockout samples. Wild-type and MSH6 knockout samples were subjected to SDS-PAGE. ab99889 and **ab18058** (loading control to Vinculin) were diluted at 1 µg/ml and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed **ab216776** secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-MSH6 antibody
[SP93] (ab99889)

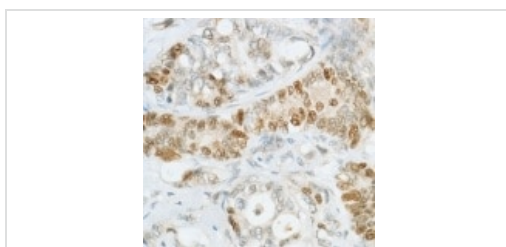
Flow cytometry analysis of HeLa (human cervix adenocarcinoma) labeling MSH6 with purified ab99889 at 1/20 dilution (5.05 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal IgG (**ab172730**) (Black). Unlabeled control - Unlabelled cells (blue).



Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [SP93] (ab99889)

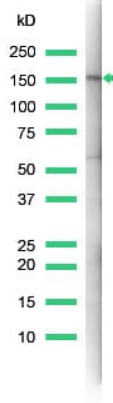
ab99889 staining MSH6 in HeLa cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab99889 at 1/250 dilution and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [SP93] (ab99889)

Staining of MSH6 in a formalin fixed, paraffin embedded Human rectal carcinoma tissue using ab99889 at a dilution of 1/100.



Anti-MSH6 antibody [SP93] (ab99889) at 1/100 dilution + Lysate prepared from A431 cells

Observed band size: 170 kDa

Western blot - Anti-MSH6 antibody [SP93]
(ab99889)

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-MSH6 antibody [SP93] (ab99889)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors