abcam

Product datasheet

Anti-MSH6 antibody [44] ab14204

KO VALIDATED

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Overview

Product name	Anti-MSH6 antibody [44]
Description	Mouse monoclonal [44] to MSH6
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide, corresponding to amino acids 225-333 of Human MSH6
Positive control	WB: HeLa, A431 and HAP1 (HAP1-MSH6 knockout cell lysate used as negative control) cell lysates. IHC-P: Human colon carcinoma tissue. ICC/IF: HeLa cells and HAP1 cells (HAP1-MSH6 knockout cells used as negative cell line).
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.3 Preservative: 0.05% Sodium azide Constituent: 1% BSA
Purity	Protein G purified
Clonality	Monoclonal
Clone number	44
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab14204 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
ICC/IF		1/250.
WB	★★★★☆ **(2)**	1/100 - 1/500.
IHC-P		Use at an assay dependent concentration.

Target

Function

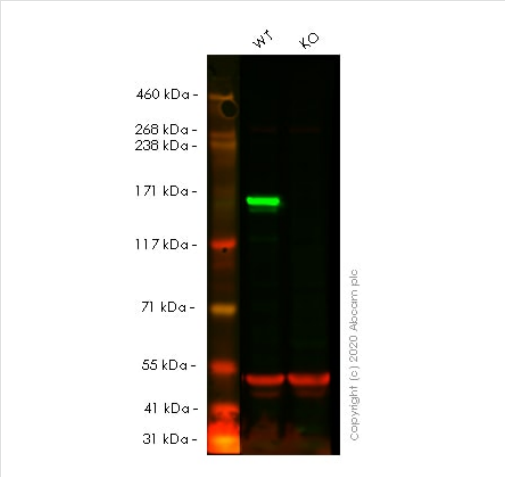
Component of the post-replicative DNA mismatch repair system (MMR). Heterodimerizes with MSH2 to form MutS alpha, which binds to DNA mismatches thereby initiating DNA repair. When bound, MutS alpha bends the DNA helix and shields approximately 20 base pairs, and recognizes single base mismatches and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



Western blot - Anti-MSH6 antibody [44] (ab14204)

All lanes : Anti-MSH6 antibody [44] (ab14204) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MSH6 knockout HeLa cell lysate

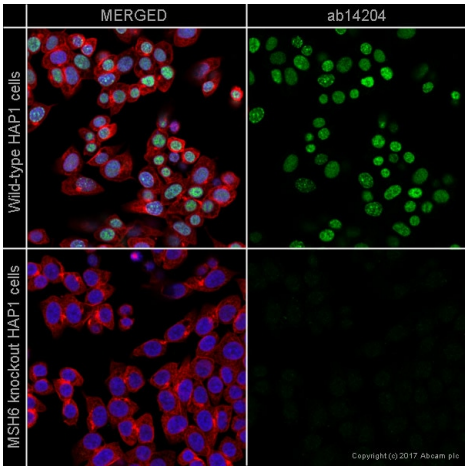
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 160 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab14204 observed at 160 kDa. Red - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (**ab52866**) observed at 50 kDa.

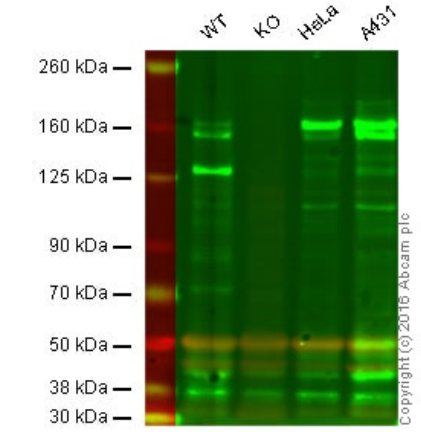
ab14204 was shown to react with MSH6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255410** (knockout cell lysate **ab263763**) was used. Wild-type HeLa and MSH6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab14204 and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (**ab52866**) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye®800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye®680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [44] (ab14204)

ab14204 staining MSH6 in wild-type HAP1 cells (top panel) and MSH6 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), 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 ab14204 at 1/250 dilution and **ab202272** 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 Mouse IgG (Alexa Fluor® 488) (**ab150117**) 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).



Western blot - Anti-MSH6 antibody [44] (ab14204)

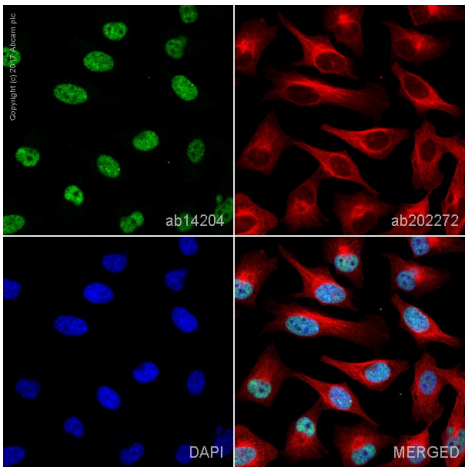
All lanes : Anti-AMACR + p63 antibody [4A4 (p63)] (**ab14202**)

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : MSH6 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : A431 cell lysate

Lysates/proteins at 20 µg per lane.

Lanes 1 - 4: Merged signal (red and green). Green - ab14204 observed at 160 kDa. Red - loading control, **ab18251**, observed at 52 kDa.

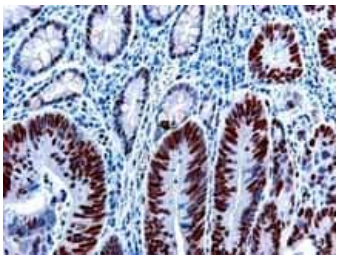
ab14204 was shown to specifically react with MSH6 in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when MSH6 knockout HAP1 samples were used. Wild-type and MSH6 knockout samples were subjected to SDS-PAGE. ab14204 and **ab18251** (loading control to alpha Tubulin) were diluted at 1/100 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [44] (ab14204)

ab14204 staining MSH6 in HeLa cells. The cells were fixed with 4% formaldehyde (10min), 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 ab14204 at 1/250dilution and **ab202272** 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 Mouse IgG (Alexa Fluor® 488) (**ab150117**) 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 [44] (ab14204)

ab14204 staining human colon carcinoma by IHC-P.

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