abcam

Product datasheet

Anti-MSH6 antibody [EPR3945] - BSA and Azide free ab214454

KO VALIDATED Recombinant RabMAb

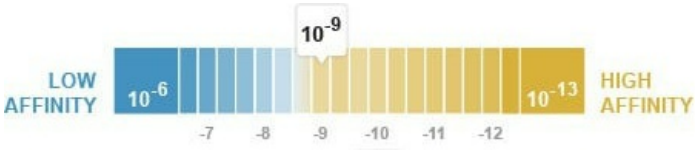
9 References 16 Images

Overview

Product name	Anti-MSH6 antibody [EPR3945] - BSA and Azide free
Description	Rabbit monoclonal [EPR3945] to MSH6 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A431, HeLa, SW480. HAP1, and HEK-293 cell lysates IHC-P: Human colonic adenocarcinoma tissue ICC/IF: HeLa cells
General notes	ab214454 is the carrier-free version of **ab92471**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 2.30 \times 10^{-9}$ M



Learn more about K_D

Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3945
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab214454 in the following tested applications.

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

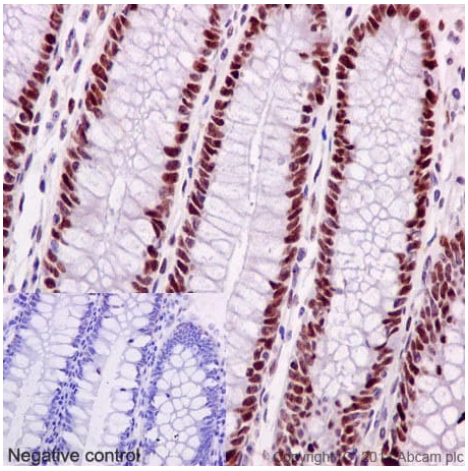
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 153 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		Use at an 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.

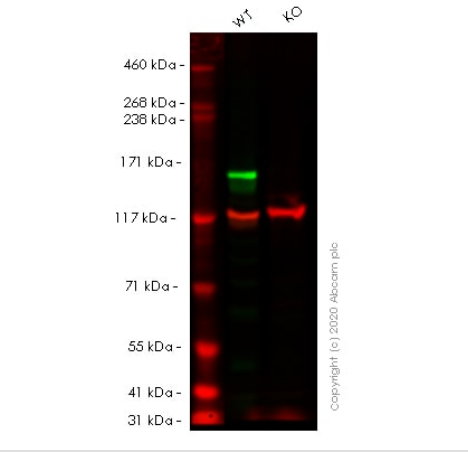
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Immunohistochemical staining of paraffin embedded human colon with unpurified **ab92471** at a dilution of 1/500. A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).



Western blot - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

All lanes : Anti-MSH6 antibody [EPR3945] (**ab92471**) at 1/1000 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.

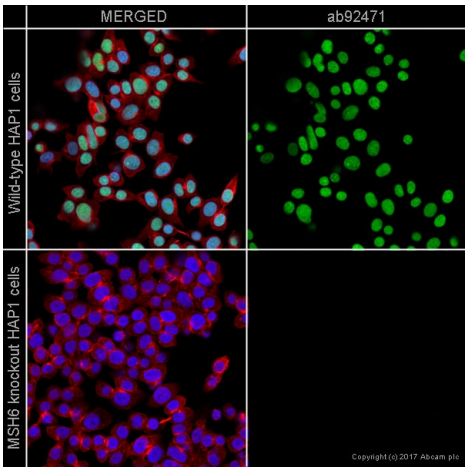
Predicted band size: 153 kDa
Observed band size: 160 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab92471**).

Lanes 1- 2: Merged signal (red and green). Green - **ab92471** observed at 160 kDa. Red - Anti-Vinculin antibody [VIN-54] observed at 124 kDa.

ab92471 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. **ab92471** and Anti-Vinculin antibody [VIN-54] overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

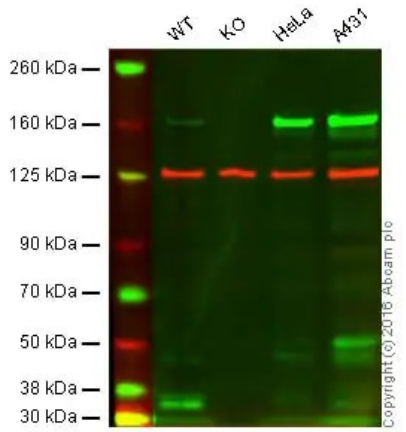


Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

ab92471 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 **ab92471** at 1µg/ml 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).



Western blot - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

All lanes : Anti-MSH6 antibody [EPR3945] (**ab92471**) at 1/1000 dilution

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.

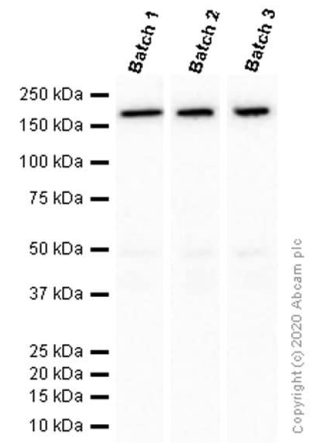
Predicted band size: 153 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab92471**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab92471** observed at 160 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

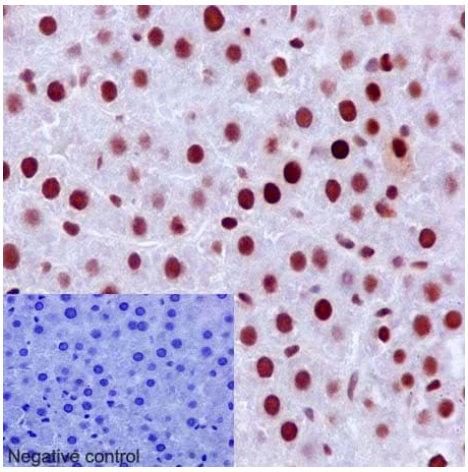
ab92471 was shown to specifically react with MSH6 in wild-type HAP1 cells. No band was observed when MSH6 knockout samples

were used. Wild-type and MSH6 knockout samples were subjected to SDS-PAGE. **ab92471** and **ab18058** (loading control to Vinculin) were diluted at 1/1000 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.



Western blot - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

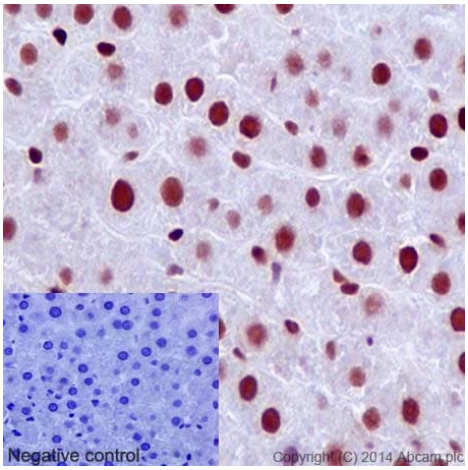
This data was developed using **ab92471**, the same antibody clone in a different buffer formulation. Different batches of **ab92471** were tested on Rat brain lysate at 0.2 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 160 kDa.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Immunohistochemical staining of paraffin embedded rat liver with purified **ab92471** at a dilution of 1/500. A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

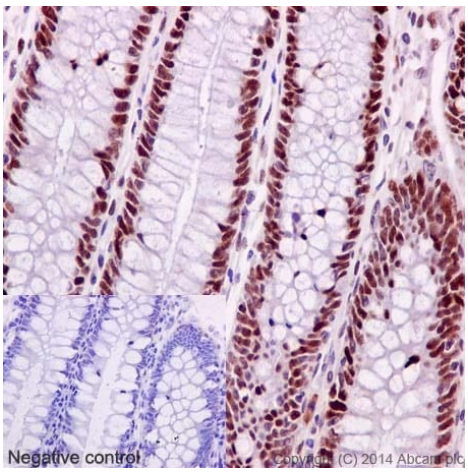
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Immunohistochemical staining of paraffin embedded rat liver with unpurified **ab92471** at a dilution of 1/150. A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

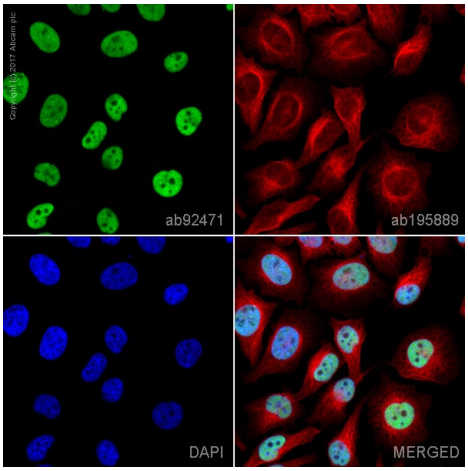
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Immunohistochemical staining of paraffin embedded human colon with unpurified **ab92471** at a dilution of 1/150. A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).

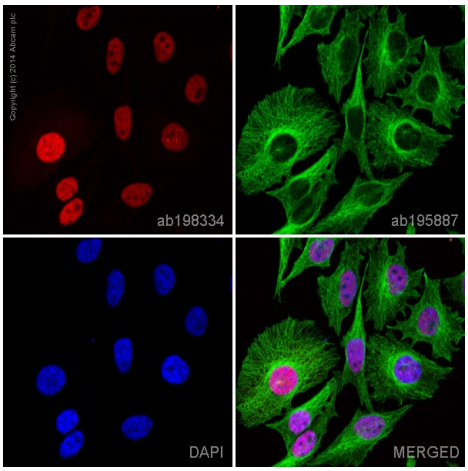


Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

ab92471 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 **ab92471** at 1µg/ml 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).



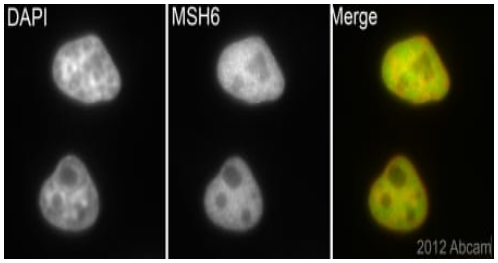
Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Clone EPR3945 (ab214454) has been successfully conjugated by Abcam. This image was generated using Anti-MSH6 antibody [EPR3945] (Alexa Fluor® 647). Please refer to **ab198334** for protocol details.

ab198334 staining MSH6 in HeLa cells. The cells were fixed with 4% formaldehyde (10 min), 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 overnight at +4°C with **ab198334** at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

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

This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5min).

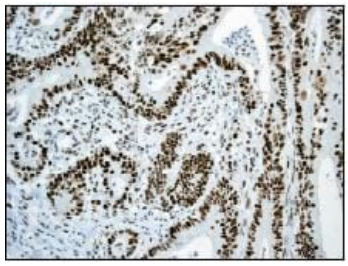


Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

This image is courtesy of an Abreview submitted by Kirk McManus.

Unpurified **ab92471** (1/500) staining MSH6 in asynchronous HeLa cells (green). Cells were fixed in paraformaldehyde, permeabilised with 0.5% Triton X100/PBS and counterstained with DAPI in order to highlight the nucleus (red). For further experimental details please see abreview.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).

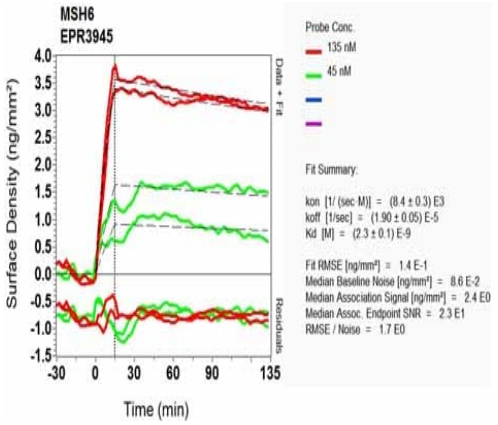


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Unpurified **ab92471**, at a 1/100 dilution, detecting MSH6 in paraffin embedded Human colonic adenocarcinoma tissue by immunohistochemistry. Detection used HRP conjugated anti rabbit antibody.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).



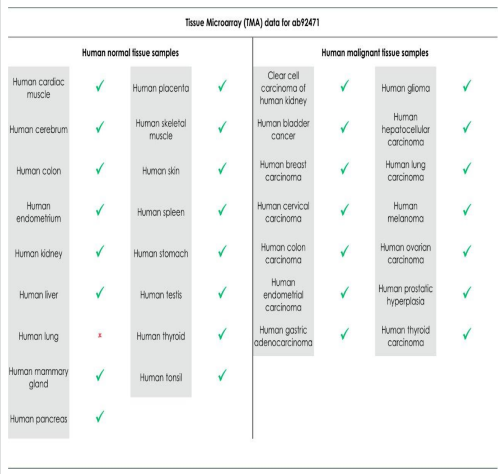
OI-RD Scanning - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92471**).

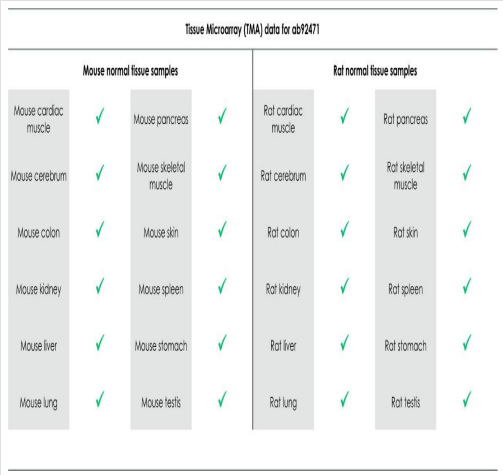


Tissue Microarray (TMA) data for ab92471

Human normal tissue samples				Human malignant tissue samples			
Human cardiac muscle	✓	Human placenta	✓	Clear cell carcinoma of human kidney	✓	Human glioma	✓
Human cerebrum	✓	Human skeletal muscle	✓	Human bladder cancer	✓	Human hepatocellular carcinoma	✓
Human colon	✓	Human skin	✓	Human breast carcinoma	✓	Human lung carcinoma	✓
Human endometrium	✓	Human spleen	✓	Human cervical carcinoma	✓	Human melanoma	✓
Human kidney	✓	Human stomach	✓	Human colon carcinoma	✓	Human ovarian carcinoma	✓
Human liver	✓	Human testis	✓	Human endometrial carcinoma	✓	Human prostatic hyperplasia	✓
Human lung	✗	Human thyroid	✓	Human gastric adenocarcinoma	✓	Human thyroid carcinoma	✓
Human mammary gland	✓	Human tonsil	✓				
Human pancreas	✓						

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Tissue Microarrays stained for " Anti-MSH6 antibody [EPR3945]" using " **ab92471**" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with **ab92471** for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

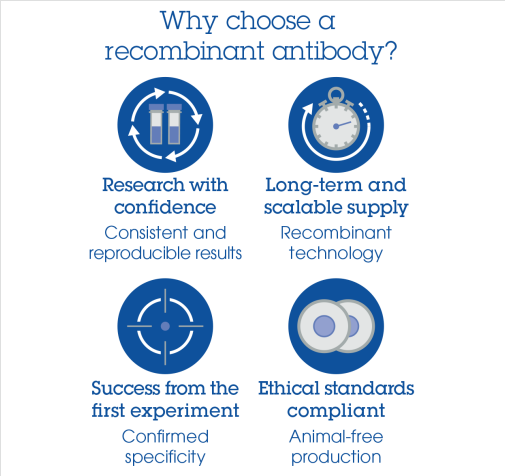


Tissue Microarray (TMA) data for ab92471

Mouse normal tissue samples				Rat normal tissue samples			
Mouse cardiac muscle	✓	Mouse pancreas	✓	Rat cardiac muscle	✓	Rat pancreas	✓
Mouse cerebrum	✓	Mouse skeletal muscle	✓	Rat cerebrum	✓	Rat skeletal muscle	✓
Mouse colon	✓	Mouse skin	✓	Rat colon	✓	Rat skin	✓
Mouse kidney	✓	Mouse spleen	✓	Rat kidney	✓	Rat spleen	✓
Mouse liver	✓	Mouse stomach	✓	Rat liver	✓	Rat stomach	✓
Mouse lung	✓	Mouse testis	✓	Rat lung	✓	Rat testis	✓

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

Tissue Microarrays stained for " Anti-MSH6 antibody [EPR3945]" using " **ab92471**" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with **ab92471** for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Anti-MSH6 antibody [EPR3945] - BSA and Azide free (ab214454)

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