

Product datasheet

Anti-MSH6 antibody [EPR3945] ab92471

KO VALIDATED Recombinant RabMAb

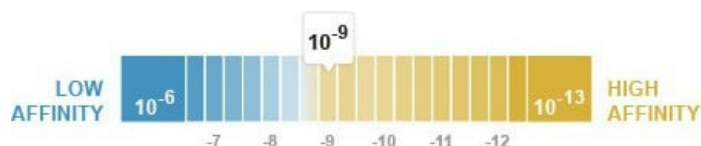
★★★★★ 5 Abreviews 52 References 17 Images

Overview

Product name	Anti-MSH6 antibody [EPR3945]
Description	Rabbit monoclonal [EPR3945] to MSH6
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human MSH6 aa 1-100 (N terminal). The exact sequence is proprietary. Database link: P52701
Positive control	WB: A431, HeLa, HAP1 and SW480 cell lysates; Rat brain lysate. IHC-P: Human colon and colonic adenocarcinoma tissue; Rat liver tissue ICC/IF: HeLa and HAP1 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>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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. Stable for 12 months at -20°C.
Dissociation constant (K_D)	K _D = 2.30 x 10 ⁻⁹ M



[Learn more about K_D](#)

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3945
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab92471 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	★★★★★ (3)	1/1000 - 1/10000. Predicted molecular weight: 153 kDa.
IHC-P	★★★★☆ (1)	1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols . For unpurified, use 1/100 - 1/250.
ICC/IF	★★★★★ (1)	Use a concentration of 1 µg/ml.

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.

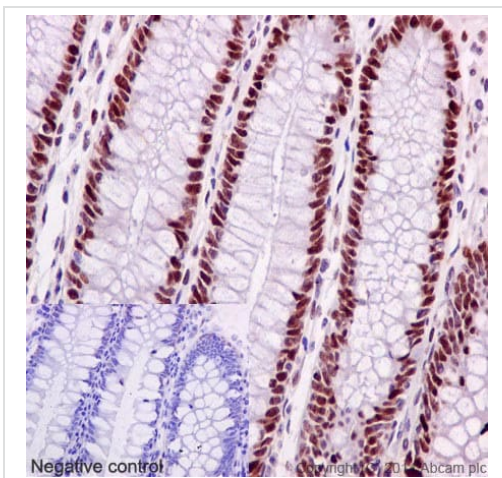
Phosphorylated upon DNA damage, probably by ATM or ATR.

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

Cellular localization

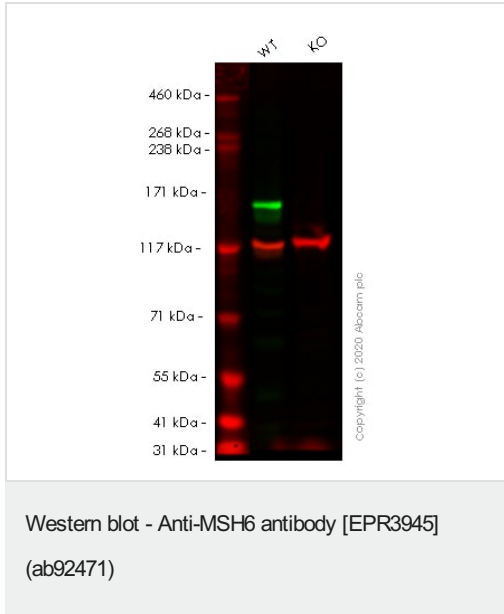
Nucleus.

Images



Immunohistochemical staining of paraffin embedded human colon 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 performed 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.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] (ab92471)



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.

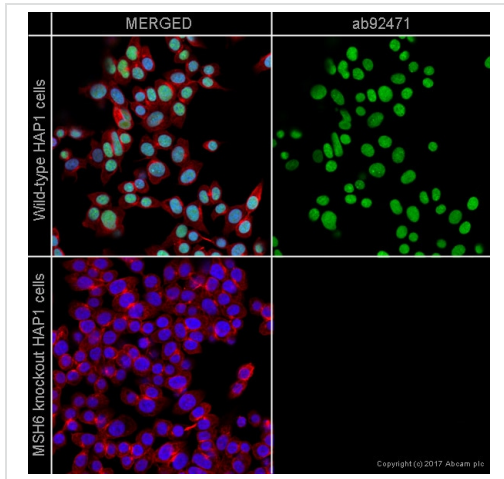
Performed under reducing conditions.

Predicted band size: 153 kDa

Observed band size: 160 kDa

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

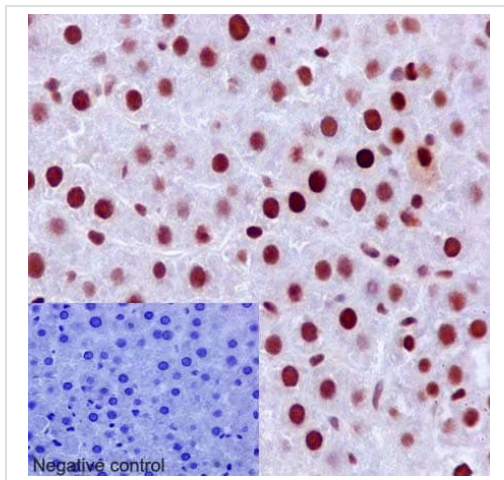
ab92471 was shown to react with MSH6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line [ab255410](#) (knockout cell lysate [ab263763](#)) was used. Wild-type HeLa and MSH6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour 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] (ab92471)

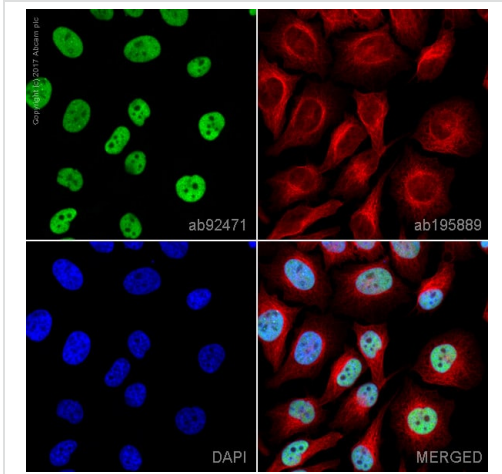
ab92471 staining MSH6 in wild-type HAP1 cells (top panel) and MSH6 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells 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).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] (ab92471)

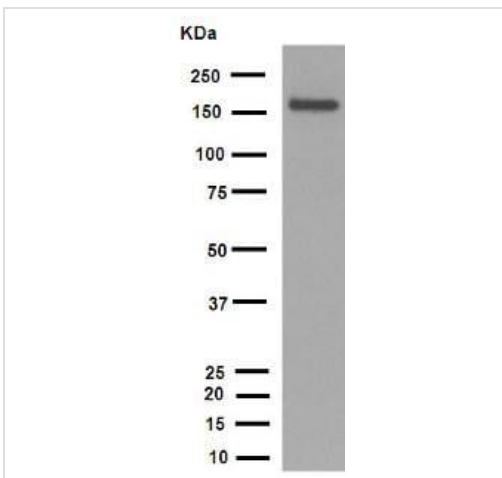
Immunohistochemical staining of paraffin embedded rat liver with purified ab92471 at a dilution of 1/500. A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.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



Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [EPR3945] (ab92471)

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).



Western blot - Anti-MSH6 antibody [EPR3945] (ab92471)

Anti-MSH6 antibody [EPR3945] (ab92471) at 1/1000 dilution (purified) + Rat brain at 10 µg

Secondary

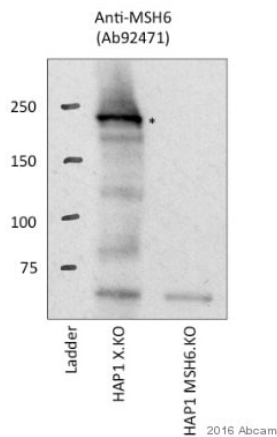
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 153 kDa

Observed band size: 160 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-MSH6 antibody [EPR3945]
(ab92471)

This image is courtesy of an Abreview by Serena Bologna.

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

Lane 1 : HAP1 WT cell lysate

Lane 2 : MSH6 KO HAP1 cell lysate

Lysates/proteins at 50 µg per lane.

Secondary

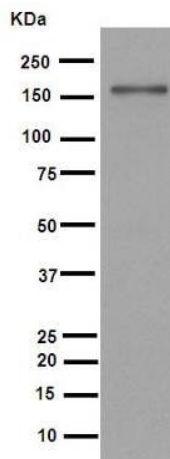
All lanes : donkey anti-rabbit IgG-HRP at 1/3000 dilution

Developed using the ECL technique.

Predicted band size: 153 kDa

Exposure time: 5 minutes

purified at 1/6000 dilution + SW480 cell lysate at 10 µg



Western blot - Anti-MSH6 antibody [EPR3945]
(ab92471)

Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 153 kDa

Observed band size: 160 kDa

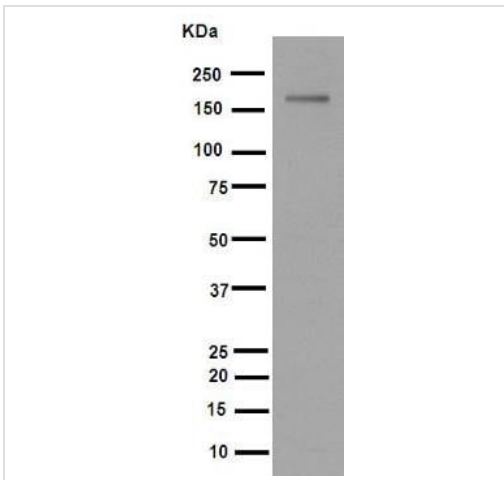
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-MSH6 antibody [EPR3945] (ab92471)

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.



Western blot - Anti-MSH6 antibody [EPR3945] (ab92471)

Anti-MSH6 antibody [EPR3945] (ab92471) at 1/2000 dilution (unpurified) + SW480 cell lysate at 10 µg

Secondary

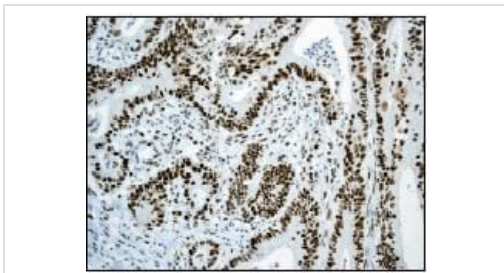
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 153 kDa

Observed band size: 160 kDa

Blocking buffer: 5% NFDM/TBST

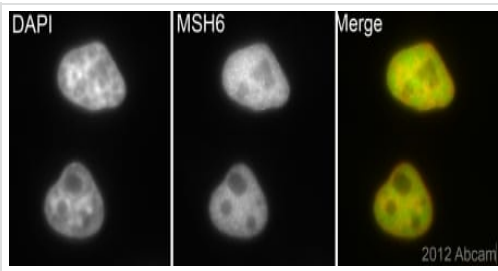
Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] (ab92471)

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.

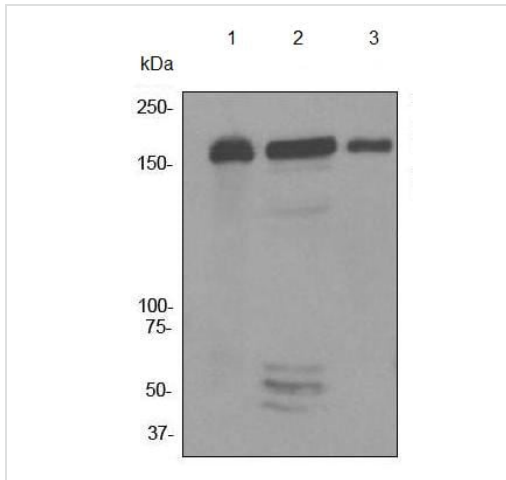
Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-MSH6 antibody [EPR3945] (ab92471)

Image courtesy of an abreview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MCB, Canada

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.



Western blot - Anti-MSH6 antibody [EPR3945] (ab92471)

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

Lane 1 : A431 cell lysate

Lane 2 : HeLa cell lysate

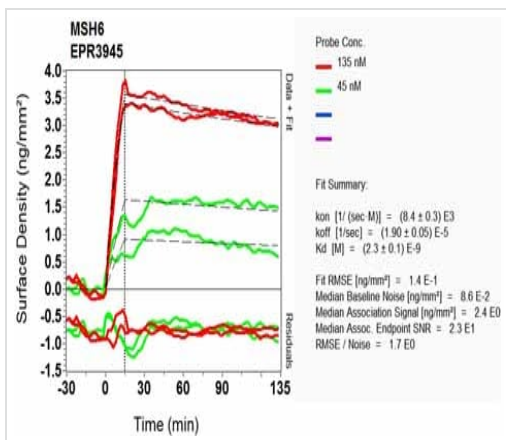
Lane 3 : SW480 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP labelled goat anti-rabbit antibody at 1/2000 dilution

Predicted band size: 153 kDa



Ox-RD Scanning - Anti-MSH6 antibody [EPR3945] (ab92471)

Secondary antibody - **goat anti-rabbit HRP (ab6721)**

Equilibrium disassociation constant (K_D)

Learn more about K_D

[Click here to learn more about \$K_D\$](#)

Tissue Microarray (TMA) data for ab92471					
Mouse normal tissue samples			Rat normal tissue samples		
Mouse cardiac muscle	✓	Mouse pancreas	✓	Rat cardiac muscle	✓
Mouse cerebrum	✓	Mouse skeletal muscle	✓	Rat cerebrum	✓
Mouse colon	✓	Mouse skin	✓	Rat colon	✓
Mouse kidney	✓	Mouse spleen	✓	Rat kidney	✓
Mouse liver	✓	Mouse stomach	✓	Rat liver	✓
Mouse lung	✓	Mouse testis	✓	Rat lung	✓

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] (ab92471)





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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MSH6 antibody [EPR3945] (ab92471)

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.

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 [EPR3945] (ab92471)

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

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