abcam

Product datasheet

Anti-MLH1 antibody [EPR3894] ab92312

KO VALIDATED Recombinant RabMAb

4 Abreviews 70 References 16 Images

Overview

Product name	Anti-MLH1 antibody [EPR3894]
Description	Rabbit monoclonal [EPR3894] to MLH1
Host species	Rabbit
Specificity	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IHC-P **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: 293, HeLa, Jurkat, A431, and SW480 cell lysates; Human testis, rat testis and mouse thymus tissue lysates. IHC-P: Human colonic adenocarcinoma and tonsil tissues. ICC/IF: HeLa and SW480 cells. Flow Cyt (intra): HeLa cells, Hap1 cells.
General notes	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: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR3894
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab92312 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)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (2)	1/2000. Predicted molecular weight: 84 kDa. **For unpurifid use at 1/10000 - 1/50000**
ICC/IF	★★★★★ (1)	1/500.
IHC-P	★★★★★ (1)	Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

Function Heterodimerizes with PMS2 to form MutL alpha, a component of the post-replicative DNA mismatch repair system (MMR). DNA repair is initiated by MutS alpha (MSH2-MSH6) or MutS beta (MSH2-MSH6) binding to a dsDNA mismatch, then MutL alpha is recruited to the heteroduplex. Assembly of the MutL-MutS-heteroduplex ternary complex in presence of RFC and PCNA is sufficient to activate endonuclease activity of PMS2. It introduces single-strand breaks near the mismatch and thus generates new entry points for the exonuclease EXO1 to degrade the strand containing the mismatch. DNA methylation would prevent cleavage and therefore assure that only the newly mutated DNA strand is going to be corrected. MutL alpha (MLH1-PMS2) interacts physically with the clamp loader subunits of DNA polymerase III, suggesting that it may play a role to recruit the DNA polymerase III to the site of the MMR. Also implicated in DNA damage signaling, a process which induces cell cycle arrest and can lead to apoptosis in case of major DNA damages. Heterodimerizes with MLH3 to form MutL gamma which plays a role in meiosis.

Tissue specificity Colon, lymphocytes, breast, lung, spleen, testis, prostate, thyroid, gall bladder and heart.

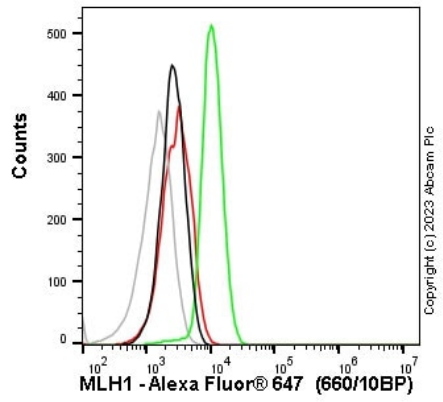
Involvement in disease Defects in MLH1 are the cause of hereditary non-polyposis colorectal cancer type 2 (HNPCC2) [MIM:609310]. Mutations in more than one gene locus can be involved alone or in combination in the production of the HNPCC phenotype (also called Lynch syndrome). Most families with clinically 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, and accounts for 15% of all colon cancers. 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.
Defects in MLH1 are a cause of mismatch repair cancer syndrome (MMRCS) [MIM:276300]; also known as Turcot syndrome or brain tumor-polyposis syndrome 1 (BTPS1). MMRCS is an autosomal dominant disorder characterized by malignant tumors of the brain associated with multiple colorectal adenomas. Skin features include sebaceous cysts, hyperpigmented and cafe au lait spots.
Defects in MLH1 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.
Note=Defects in MLH1 may contribute to lobular carcinoma in situ (LCIS), a non-invasive neoplastic disease of the breast.
Defects in MLH1 are a cause of susceptibility to endometrial cancer (ENDMC) [MIM:608089].
Note=Some epigenetic changes can be transmitted unchanged through the germline (termed 'epigenetic inheritance'). Evidence that this mechanism occurs in humans is provided by the identification of individuals in whom 1 allele of the MLH1 gene is epigenetically silenced throughout the soma (implying a germline event). These individuals are affected by HNPCC but does not have identifiable mutations in MLH1, even though it is silenced, which demonstrates that an epimutation can phenocopy a genetic disease.

Sequence similarities Belongs to the DNA mismatch repair mutL/hexB family.

Cellular localization Nucleus.

Images



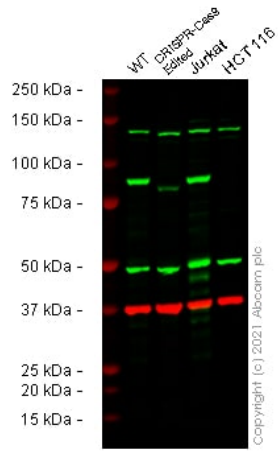
Flow Cytometry (Intracellular) - Anti-MLH1 antibody [EPR3894] (ab92312)

Flow cytometry overlay histogram showing wild-type Hap1 (green line) and MLH1 knockout Hap1 stained with ab92312 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab92312) (1x 10^6 in 100µl at 0.2 µg/ml (1/9835)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, MLH1 knockout Hap1 - grey line). Unlabelled sample was also used

as a control (this line is not shown for the purpose of simplicity).



Western blot - Anti-MLH1 antibody [EPR3894] (ab92312)

All lanes : Anti-MLH1 antibody [EPR3894] (ab92312) at 1/2000 dilution

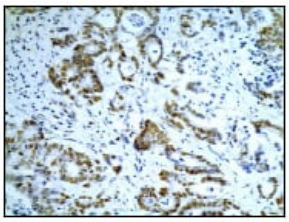
Lane 1 : Wild-type A549 cell lysate
Lane 2 : MLH1 CRISPR-Cas9 edited A549 cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HCT 116 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

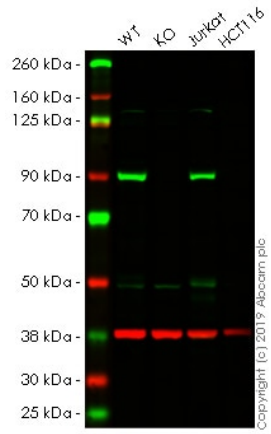
Predicted band size: 84 kDa
Observed band size: 85 kDa

False colour image of Western blot: Anti-MLH1 antibody [EPR3894] staining at 1/2000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab92312 was shown to bind specifically to MLH1. A band was observed at 85 kDa in wild-type A549 cell lysates with no signal observed at this size in MLH1 CRISPR-Cas9 edited cell line **ab276105** (CRISPR-Cas9 edited cell lysate **ab283566**). The band observed in the CRISPR-Cas9 edited lysate lane below 85 kDa is likely to represent a truncated form of MLH1. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and MLH1 CRISPR-Cas9 edited A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR3894] (ab92312)

Unpurified ab92312 at 1/100 dilution staining MLH1 in Human colonic adenocarcinoma by Immunohistochemistry, Paraffin-embedded tissue. The use of an HRP/AP polymerized antibody is recommended for a secondary antibody. Heat mediated antigen retrieval was performed via the pressure cooker method before commencing with IHC staining protocol.



Western blot - Anti-MLH1 antibody [EPR3894] (ab92312)

All lanes : Anti-MLH1 antibody [EPR3894] (ab92312) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MLH1 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HCT116 cell lysate

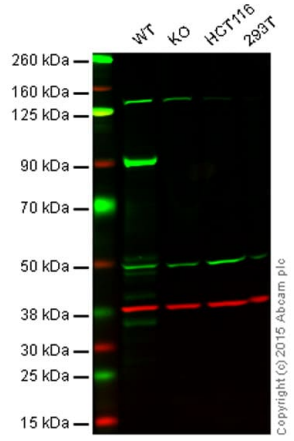
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 84 kDa
Observed band size: 85 kDa

Lanes 1-4: Merged signal (red and green). Green - ab92312 observed at 90 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab92312 Anti-MLH1 antibody [EPR3894] was shown to specifically react with MLH1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab267223** (knockout cell lysate **ab257172**) was used. Wild-type and MLH1 knockout samples were subjected to SDS-PAGE. ab92312 and Anti-GAPDH antibody [6C5] - Loading Control were incubated overnight at 4°C at 1 in 10000 dilution and 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.



Western blot - Anti-MLH1 antibody [EPR3894] (ab92312)

All lanes : Anti-MLH1 antibody [EPR3894] (ab92312) at 1/1000 dilution

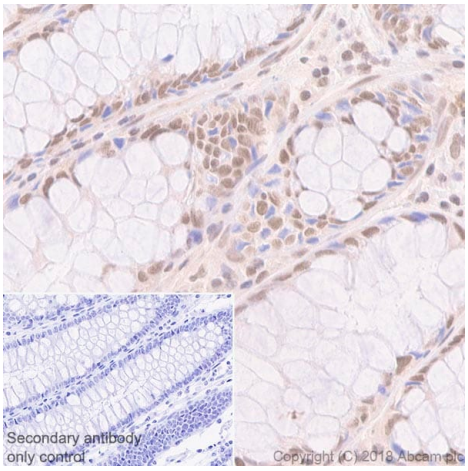
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : MLH1 knockout HAP1 cell lysate
Lane 3 : HCT116 cell lysate
Lane 4 : 293T cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 84 kDa

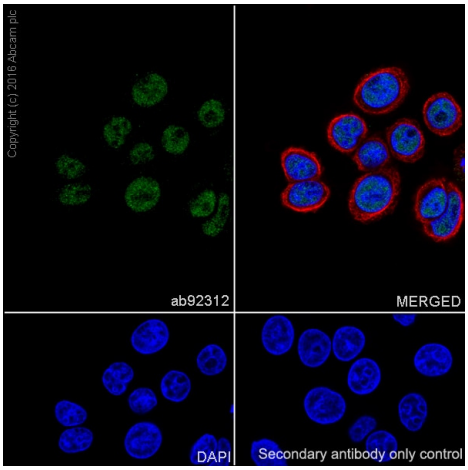
Lanes 1 - 4: Merged signal (red and green). Green - ab92312 observed at 88 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

Unpurified ab92312 was shown to recognize MLH1 in wild-type HAP1 cells along with additonal cross reactive bands. No band was observed when MLH1 knockout samples were examined. Wild-type and MLH1 knockout samples were subjected to SDS-PAGE. ab92312 and **ab8245** (loading control to GAPDH) were both diluted 1/1000 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 hour at room temperature before imaging.



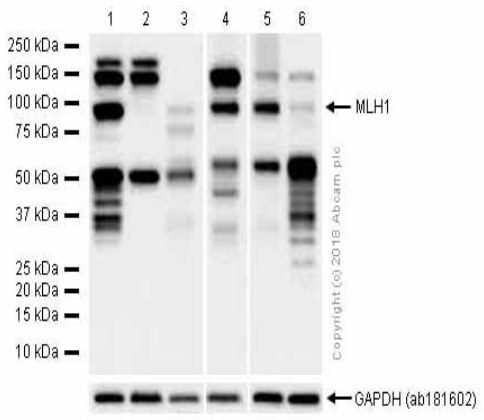
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR3894] (ab92312)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon tissue sections labeling MLH1 with Purified ab92312 at 1:250 dilution (2.9 µg/ml). Heat mediated antigen retrieval was performed using citrate (pH 6.0)ImmunoHistoProbe one step HRP Polymer (ready to use)was used as the secondary antibody.Negative control:PBS instead of the primary antibody.Hematoxylinwas used as a counterstain



Immunocytochemistry/ Immunofluorescence - Anti-MLH1 antibody [EPR3894] (ab92312)

Immunocytochemistry/ Immunofluorescence analysis of SW480 (Human colorectal adenocarcinoma epithelial cell) cells labeling MLH1 with Purified ab92312 at 1:500 dilution (1.6 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor®594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor®488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-MLH1 antibody [EPR3894] (ab92312)

All lanes : Anti-MLH1 antibody [EPR3894] (ab92312) at 0.4 µg/ml (purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HCT116 (Human colorectal carcinoma epithelial cell) whole cell lysates, negative control
Lane 3 : Human testis lysates
Lane 4 : Rat testis lysates
Lane 5 : Mouse thymus lysates
Lane 6 : Rat thymus lysates

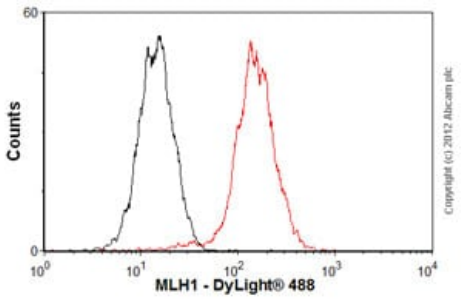
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

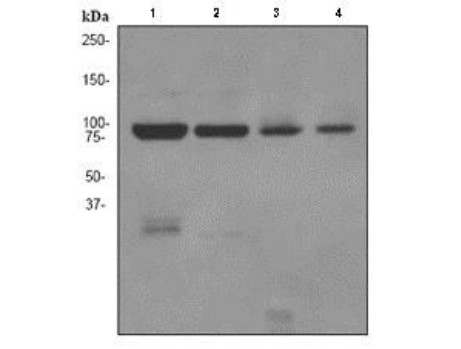
Predicted band size: 84 kDa

Blocking and diluting buffer: 5% NFDM/TBST.
According to PMID:23653048, HCT116 is MLH1 negative cell line.



Flow Cytometry (Intracellular) - Anti-MLH1 antibody [EPR3894] (ab92312)

Overlay histogram showing HeLa cells stained with unpurifiedab92312 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab92312, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-MLH1 antibody [EPR3894] (ab92312)

All lanes : Anti-MLH1 antibody [EPR3894] (ab92312) at 1/10000 dilution (Unpurified)

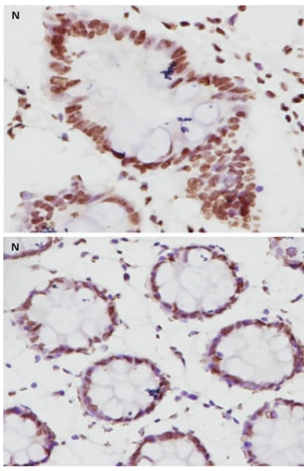
Lane 1 : 293 cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : A431 cell lysate
Lane 4 : SW480 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

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

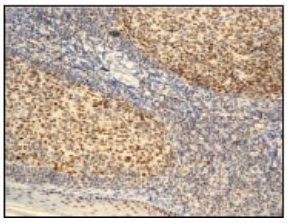
Predicted band size: 84 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR3894] (ab92312)

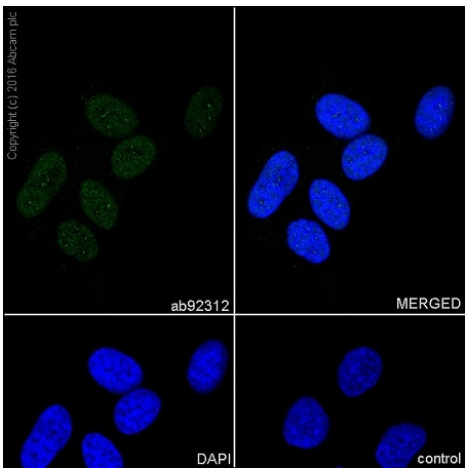
Image from Wang X et al., PLoS One. 2011;6(10):e25913. Epub 2011 Oct 12. Fig 3.; doi:10.1371/journal.pone.0025913; October 12, 2011, PLoS ONE 6(10): e25913.

Unpurified ab92312 staining MLH1 in Human colorectal (top) and gastric tissue (bottom) by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR3894] (ab92312)

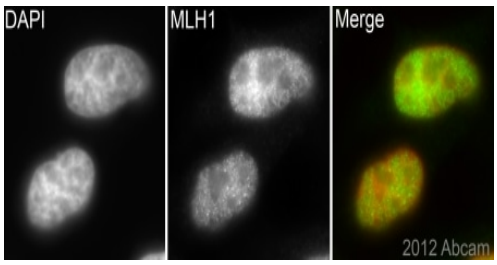
Unpurified ab92312 at 1/100 dilution staining MLH1 in Human tonsil by Immunohistochemistry, Paraffin-embedded tissue. The use of an HRP/AP polymerized antibody is recommended for a secondary antibody. Heat mediated antigen retrieval was performed via the pressure cooker method before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-MLH1 antibody [EPR3894] (ab92312)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) labelling MLH1 with purified ab92312 at 1/1000. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

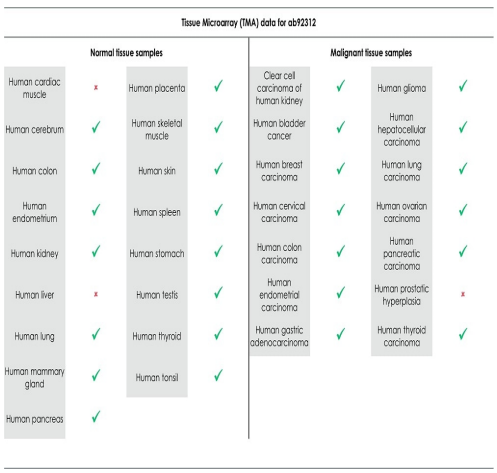
Control: PBS only



Immunocytochemistry/ Immunofluorescence - Anti-MLH1 antibody [EPR3894] (ab92312)

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

Unpurified ab92312 (1/200) staining MLH1 in 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 refer to abreview.



Tissue Microarray (TMA) data for ab92312

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x	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 ovarian carcinoma	✓
Human kidney	✓	Human stomach	✓	Human colon carcinoma	✓	Human pancreatic carcinoma	✓
Human liver	x	Human testis	✓	Human endometrial carcinoma	✓	Human prostatic hyperplasia	x
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-MLH1 antibody [EPR3894] (ab92312)

Tissue Microarrays stained for "Anti-MLH1 antibody [EPR3894]" using "ab92312" 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 **ab93678** (citrate buffer, pH 6.0). The sections were incubated with ab92312 at +4°C overnight. ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody.

Anti-MLH1 antibody [EPR3894] (ab92312)

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