abcam

ab92312

Anti-MLH1 antibody [EPR3894]

Anti-MLH1 antibody [EPR3894] (ab92312) is a rabbit monoclonal antibody detecting MLH1 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 80 publications
- Trusted since 2010

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR3894
Purification technique	Affinity purification Protein A
Concentration	0.747 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/2000
Notes	**For unpurifid use at 1/10000 - 1/50000**

Species	Rat
Dilution info	1/2000
Notes	**For unpurifid use at 1/10000 - 1/50000**

Species	Human
Dilution info	1/2000
Notes	**For unpurifid use at 1/10000 - 1/50000**

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information MLH1

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-MSH3) 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.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-MLH1 antibody [EPR3894] (ab92312) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse, Rat samples.

What is the molecular weight of MLH1?
Anti-MLH1 [EPR3894] (ab92312) specifically detects a band for MLH1 (UniProt: P40692) at a molecular weight of 84kDa.

Trusted by the scientific community
Anti-MLH1 [EPR3894] (ab92312) was first used in a scientific publication in 2010 and has been cited over 80 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-MLH1 antibody [EPR3894] (ab92312) has been confirmed by Western blot testing in MLH1 Knockout HAP1 cell line, ab276105.

Other related products
We have a range of other formats of antibody clone [EPR3894] also available for your convenience: ab92312, Alexa Fluor® 488 - ab199237, Alexa Fluor® 647 - ab199494, Carrier free - ab214441, Alexa Fluor® 555 - ab215303, PE - ab303047, APC - ab303048, HRP - ab303049, Alkaline Phosphatase - ab308849, Alexa Fluor® 594 - ab310577, Alexa Fluor® 568 - ab312589, Alexa Fluor® 750 - ab321745

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	MLH1 also known as MutL homolog 1 is a protein involved in DNA mismatch repair an important mechanism for maintaining genetic stability. It has a molecular weight of approximately 87 kDa. This protein is expressed in various tissues but is most abundant in the colonic epithelium and endometrium. MLH1 acts mechanically by forming heterodimers with other proteins collaborating in correcting errors that occur during DNA replication.
Biological function summary	The function of MLH1 involves its role in the mismatch repair (MMR) system. It is part of a complex with PMS2 forming a heterodimer known as MutLα which is essential for the repair process. This complex scans newly synthesized DNA for mispaired bases and initiates repair preserving genomic integrity. The proper function of MLH1 and its interaction with PMS2 ensures that DNA replication errors do not accumulate and cause harmful mutations.
Pathways	MLH1 operates within the mismatch repair pathway and interacts closely with MLH3 and PMS2 proteins. It plays a critical role in the recognition and repair of mismatched bases that occur during DNA replication particularly in the G2 phase of the cell cycle. Through its involvement in the mismatch repair pathway MLH1 is connected to cell cycle regulation and the DNA damage response pathway.
Associated diseases and disorders	MLH1 mutations are closely linked to Lynch syndrome and sporadic colorectal cancer. Lynch syndrome a hereditary condition significantly raises the risk of colorectal cancer and other cancers due to defective DNA mismatch repair. MLH1 mutations often lead to the loss of MLH1 protein expression particularly observed in MLH1 IHC staining. Additionally in colorectal cancer the MLH1 protein may interact with APC and TP53 playing a role in cancer progression and tumorigenesis.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

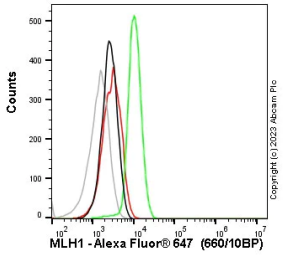
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

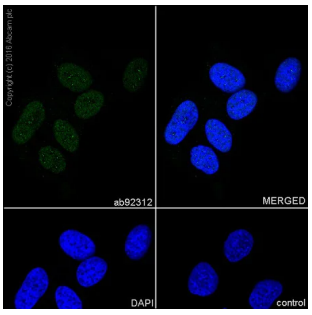
Full details and terms and conditions can be found here:
Terms & Conditions.

16 product images



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

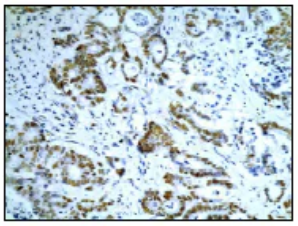
Flow cytometry overlay histogram showing wild-type Hap1 (green line) and MLH1 knockout Hap1 stained with ab92312 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab92312) (1x 106 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).



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

MLH1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MLH1 antibody

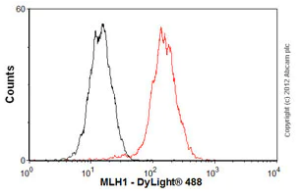
Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) labelling MLH1 with purified ab92312 at 1/1000. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (ab150077). Nuclei counterstained with DAPI (blue).
Control: PBS only



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

MLH1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MLH1 antibody

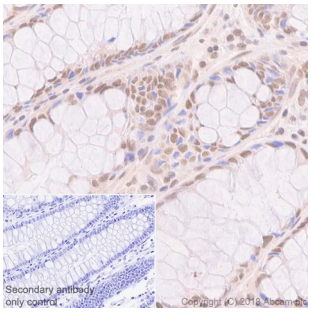
Unpurified ab92312 at 1/100 dilution staining MLH1 in Human colonic adenocarcinoma by Immunohistochemistry, Paraffin-embedded tissue. The use of an HRP/AP polymerized antibody is recommended for a secondary antibody. Heat mediated antigen retrieval was performed via the pressure cooker method before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



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

MLH1 Flow Cytometry (Intracellular) staining using rabbit Anti-MLH1 antibody

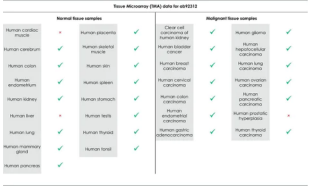
Overlay histogram showing HeLa cells stained with unpurifiedab92312 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions 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.



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

MLH1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MLH1 antibody

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



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.

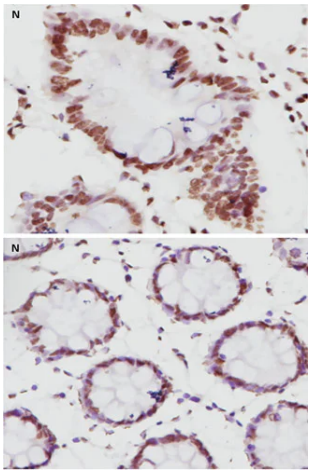
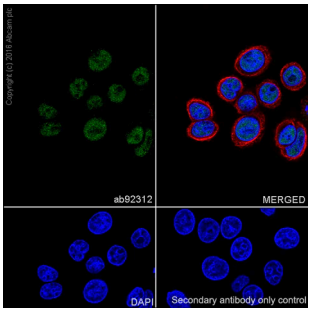


Image from Wang X et al. PLoS One. 2011;6(10):e25913. Epub 2011 Oct 12. Fig 3.; doi:10.1371/journal.pone.0025913; October 12 2011 PLoS ONE 6(10): e25913.

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

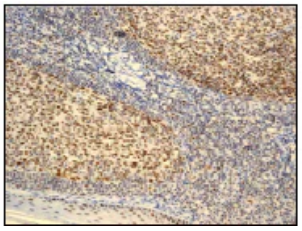
Unpurified ab92312 staining MLH1 in Human colorectal (top) and gastric tissue (bottom) by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



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

MLH1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MLH1 antibody

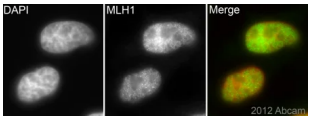
Immunocytochemistry/ Immunofluorescence analysis of SW480 (Human colorectal adenocarcinoma epithelial cell) cells labeling MLH1 with Purified ab92312 at 1:500 dilution (1.6 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 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.



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

MLH1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MLH1 antibody

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.

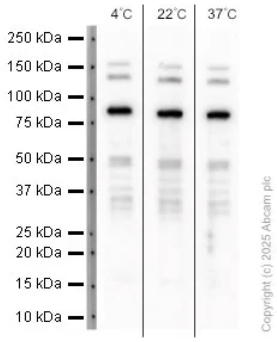


This image is courtesy of a customer review submitted by Kirk McManus.

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

MLH1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MLH1 antibody

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.



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

MLH1 Western blot staining of HeLa whole cell lysate using rabbit Anti-MLH1 antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of HeLa whole cell lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab92312 (1/10,000) at room temperature for 1hr. After being washed three times in TBST, the membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

ab92312 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.

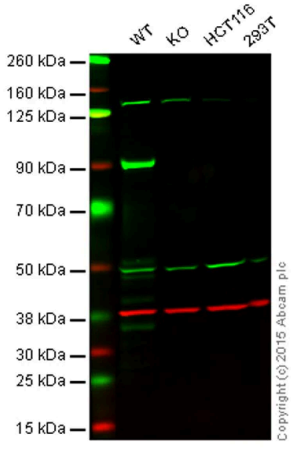
All lanes:
Western blot - Anti-MLH1 antibody [EPR3894] (ab92312) at 1/10000 dilution

All lanes:
HeLa whole cell lysate at 15 µg with NDFM/TBST

Secondary

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

Exposure time: 20s



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

MLH1 Western blot staining using rabbit Anti-MLH1 antibody

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.

All lanes:

Western blot - Anti-MLH1 antibody [EPR3894] (ab92312) at 1/1000 dilution

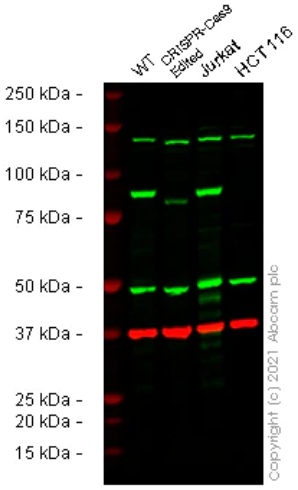
Lane 1:
Wild-type HAP1 cell lysate at 20 µg

Lane 2:
MLH1 knockout HAP1 cell lysate at 20 µg

Lane 3:
HCT116 cell lysate at 20 µg

Lane 4:
293T cell lysate at 20 µg

Predicted band size: 85 kDa



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

MLH1 Western blot staining using rabbit Anti-MLH1 antibody

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

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
MLH1 CRISPR-Cas9 edited A549 cell lysate at 20 µg

Lane 2:
Western blot - Human MLH1 knockout A549 cell line (ab276105)

Lane 2:
Western blot - Human MLH1 knockout A549 cell lysate (ab288239)

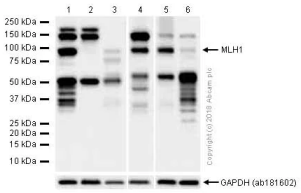
Lane 3:
Jurkat cell lysate at 20 µg

Lane 4:
HCT 116 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 85 kDa

Observed band size: 85 kDa



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

Blocking and diluting buffer: 5% NFDM/TBST.
According to PMID:23653048, HCT116 is MLH1 negative cell line.
All lanes:
Western blot - Anti-MLH1 antibody [EPR3894] (ab92312) at 0.4 µg/mL

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Lane 2:
HCT116 (Human colorectal carcinoma epithelial cell) whole cell lysates, negative control at 20 µg

Lane 3:
Human testis lysates at 20 µg

Lane 4:
Rat testis lysates at 20 µg

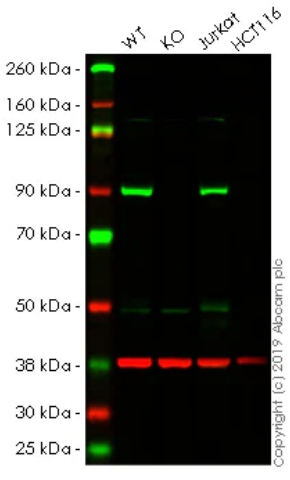
Lane 5:
Mouse thymus lysates at 20 µg

Lane 6:
Rat thymus lysates at 20 µg

Secondary

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

Predicted band size: 85 kDa



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

MLH1 Western blot staining using rabbit Anti-MLH1 antibody

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.

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

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human MLH1 knockout HeLa cell lysate (ab257172) at 20 µg

Lane 3:

Jurkat cell lysate at 20 µg

Lane 4:
HCT116 cell lysate at 20 µg

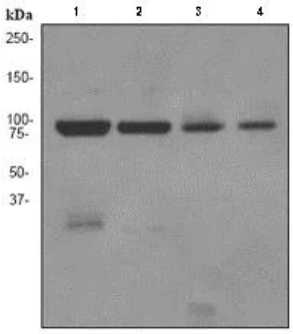
Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 85 kDa

Observed band size: 85 kDa



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

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

Lane 1:
293 cell lysate at 10 µg

Lane 2:
HeLa cell lysate at 10 µg

Lane 3:
A431 cell lysate at 10 µg

Lane 4:
SW480 cell lysate at 10 µg

Secondary

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

Predicted band size: 85 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.