abcam

ab315011

Anti-MLH1 antibody [EPR26314-1]

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	EPR26314-1
Purification technique	Affinity purification Protein A
Concentration	0.508 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse

Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/200
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Mouse
Dilution info	1/500
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/500
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Expected

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

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
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 (By similarity).

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

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.

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

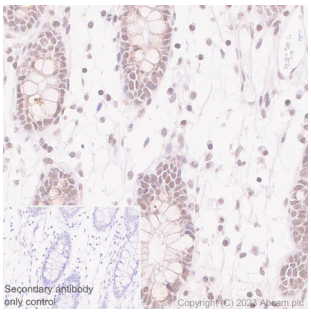
We do not recommend this combination. It is not covered by our product promise.

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.

12 product images

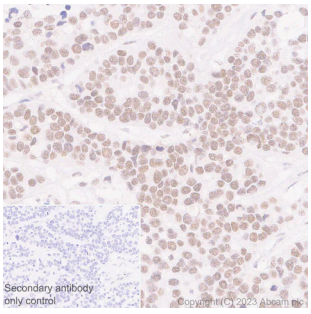


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling MLH1 with ab315011 at 1/200 (2.54 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human colon. The section was incubated with ab315011 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

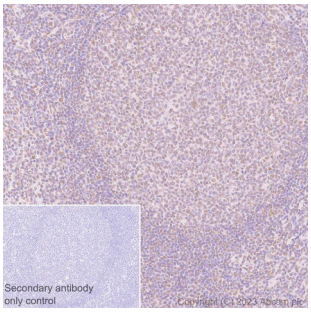


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Immunohistochemical analysis of paraffin-embedded Human gastric adenocarcinoma tissue labeling MLH1 with ab315011 at 1/200 (2.54 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human gastric adenocarcinoma. The section was incubated with ab315011 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

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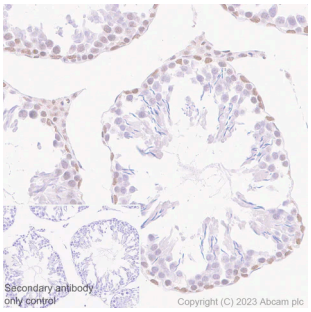


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling MLH1 with ab315011 at 1/200 (2.54 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human tonsil. The section was incubated with ab315011 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

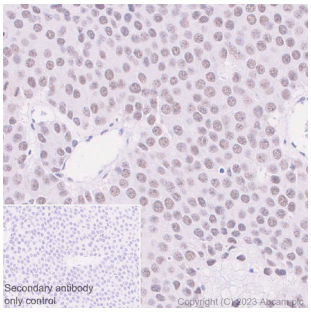


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling MLH1 with ab315011 at 1/500 (1.016 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on rat testis. The section was incubated with ab315011 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

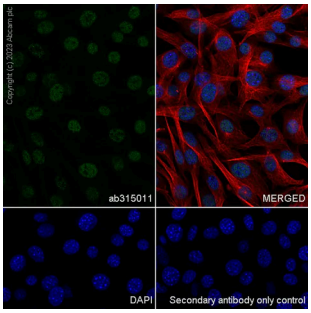


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Immunohistochemical analysis of paraffin-embedded Mouse breast cancer tissue labeling MLH1 with ab315011 at 1/500 (1.016 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse breast cancer. The section was incubated with ab315011 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

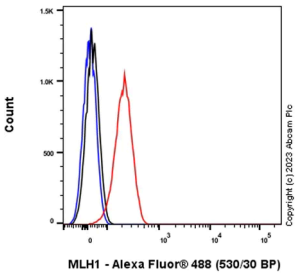


Immunocytochemistry/ Immunofluorescence - Anti-MLH1 antibody [EPR26314-1] (ab315011)

MLH1 Immunocytochemistry/ Immunofluorescence staining of NIH/3T3 (mouse embryonic fibroblast) using rabbit Anti-MLH1 antibody

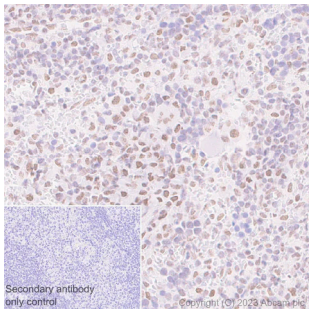
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling MLH1 with ab315011 at 1/50 (10.16 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/mL) dilution (Green). Confocal image showing nuclear staining in NIH/3T3 cell line. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/mL)dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution.



Flow Cytometry (Intracellular) - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling MLH1 with ab315011 at 1/50 dilution (1 ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

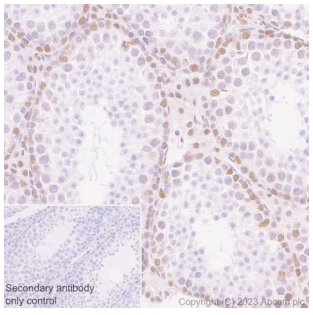


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling MLH1 with ab315011 at 1/500 (1.016 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on rat spleen. The section was incubated with ab315011 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

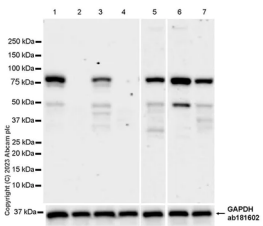


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling MLH1 with ab315011 at 1/500 (1.016 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse testis. The section was incubated with ab315011 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Western blot - Anti-MLH1 antibody [EPR26314-1] (ab315011)

MLH1 Western blot staining using rabbit Anti-MLH1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: HCT 116 (PMID:23653048).

In Western blot, ab315011 was shown to bind specifically to MLH1. A band was observed at 84 kDa in wild-type Hela cell lysates with whereas no signal observed at this size in MLH1 knockout cell line ab267223 (knockout cell lysate ab257172).

The identity of the lower MW bands below 50kDa are unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

Exposure time: Lanes 1-4: 59 seconds; lanes 5-7: 180 seconds.
All lanes:
Western blot - Anti-MLH1 antibody [EPR26314-1] (ab315011) at 1/1000 dilution

Lane 1:
Wild-type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
MLH1 knockout HeLa whole cell lysate at 20 µg

Lane 3:
Jurkat (human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

Lane 4:
HCT116 (human colorectal carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
C2C12 (mouse myoblasts myoblast) whole cell lysate at 20 µg

Lane 6:
ES-D3 (mouse embryonic pluripotent stem Cell) whole cell lysate at 20 µg

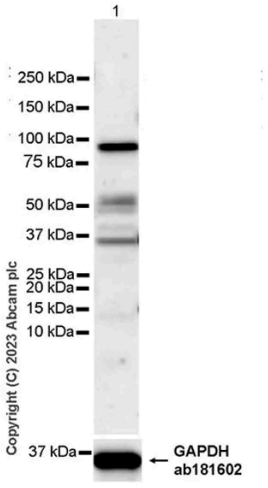
Lane 7:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Secondary

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

Performed under reducing conditions.

Observed band size: 84 kDa, 36 kDa



Western blot - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Lysate was freshly made and used for Western blotting immediately to minimize protein degradation.

The identity of the lower MW bands below 50kDa are unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-MLH1 antibody [EPR26314-1] (ab315011) at 1/1000 dilution

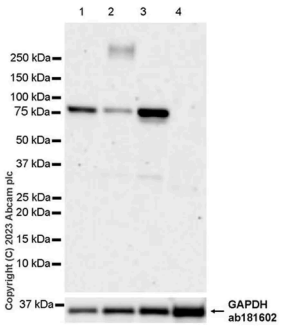
All lanes:
Rat spleen tissue lysate at 20 µg

Secondary

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

Observed band size: 84 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-MLH1 antibody [EPR26314-1] (ab315011)

Blocking and diluting buffer and concentration: 5% NFDM/TBST,

Low expression: skeletal muscle.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-MLH1 antibody [EPR26314-1] (ab315011) at 1/1000 dilution

Lane 1:
Mouse testis tissue lysate at 20 µg

Lane 2:
Mouse colon tissue lysate at 20 µg

Lane 3:
Mouse thymus tissue lysate at 20 µg

Lane 4:
Mouse skeletal muscle tissue lysate at 20 µg

Secondary

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

Observed band size: 84 kDa, 36 kDa

Exposure time: 180s

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