abcam

Product datasheet

Anti-MLH1 antibody [EPR20522] ab223844

KO 評価済　リコンビナント　RabMAb

1 References　画像数 13

製品の概要

製品名	Anti-MLH1 antibody [EPR20522]
製品の詳細	Rabbit monoclonal [EPR20522] to MLH1
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: SW480 and HeLa whole cell lysates; human colon tissue lysate. IHC-P: Human colon, colon cancer and ovarian cancer tissues, human breast tissue ICC/IF: Hap1 (MLH1 knockout Hap1 cells used as a negative control) cells. Flow cyto (intra): SW480 cells, Hap1 cells. IP: HeLa whole cell lysate.
特記事項	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**.

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**.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified

ポリ/モノ	モノクローナル
クローン名	EPR20522
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab223844の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/600.
WB		1/1000. Detects a band of approximately 84 kDa (predicted molecular weight: 84 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use a concentration of 0.2 µg/ml. This product gave a positive signal in Hap1 (MLH1 knockout Hap1 cells used as a negative control) fixed with 4% formaldehyde (10 min).
IP		1/30.

ターゲット情報

機能	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.
組織特異性	Colon, lymphocytes, breast, lung, spleen, testis, prostate, thyroid, gall bladder and heart.
関連疾患	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.
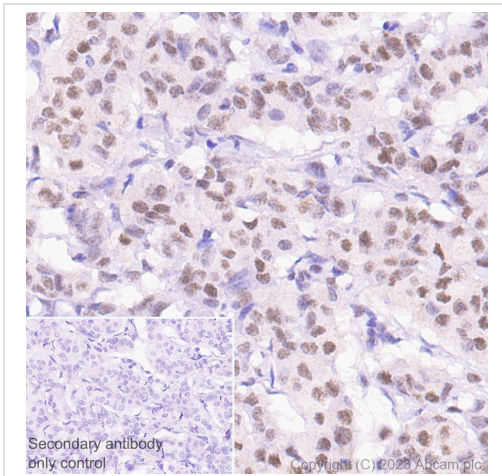
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配列類似性	Belongs to the DNA mismatch repair mutL/hexB family.
細胞内局在	Nucleus.

画像

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR20522] (ab223844)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labelling MLH1 with ab223844 at 1/100 (7.79 µg/ml) dilution for 10 minutes at room temperature, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) ready to use. Positive staining on human breast carcinoma is observed. Counter stained with hematoxylin.The immunostaining was performed on a Leica Biosystems BOND® RX instrument

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

Perform heat mediated antigen retrieval with Tris/EDTA buffer (pH 9.0,Epitope Retrieval Solution2) for 10 minutes before commencing with IHC staining protocol.
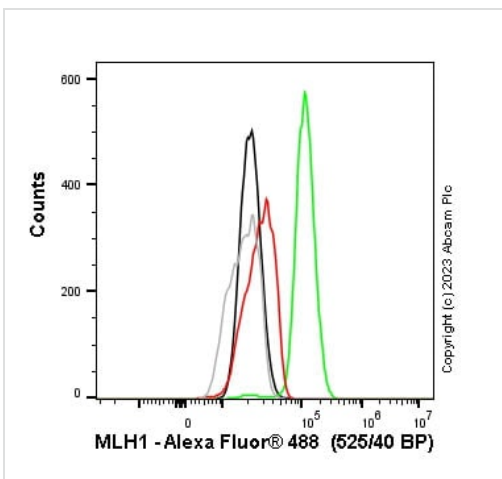

Flow Cytometry (Intracellular) - Anti-MLH1 antibody [EPR20522] (ab223844)

Flow cytometry overlay histogram showing wild-type Hap1 (green line) and MLH1 knockout Hap1 stained with ab223844 (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 (ab223844) (1x 10^6 in 100µl at 1.0 µg/ml (1/2070)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) 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).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

Western blot - Anti-MLH1 antibody [EPR20522] (ab223844)
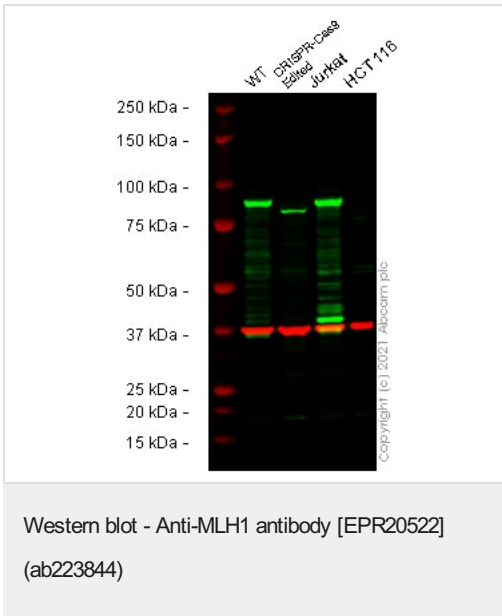
All lanes : Anti-MLH1 antibody [EPR20522] (ab223844) at 1/1000 dilution

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 [EPR20522] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab223844 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.
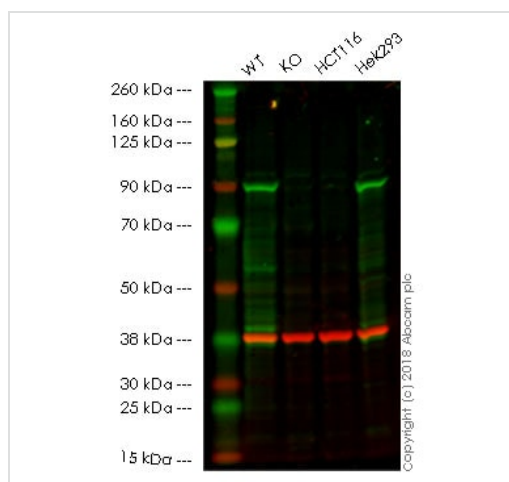
Western blot - Anti-MLH1 antibody [EPR20522] (ab223844)

All lanes : Anti-MLH1 antibody [EPR20522] (ab223844) at 1 µg/ml

Lane 1 : Wild-type HAP1 whole cell lysate

**Lane 2 :** MLH1 knockout HAP1 whole cell lysate

Lane 3 : HCT116 whole cell lysate

Lane 4 : Hek293 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 84 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab223844 observed at 85 kDa. Red - loading control, **ab9484**, observed at 37 kDa

ab223844 was shown to specifically react with MLH1 in wild-type HAP1 cells as signal was lost in MLH1 knockout cells. Wild-type and MLH1 knockout samples were subjected to SDS-PAGE. Ab223844 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.
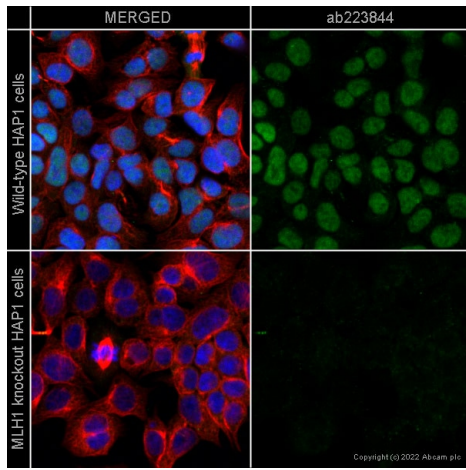
Immunocytochemistry/ Immunofluorescence - Anti-MLH1 antibody [EPR20522] (ab223844)

ab223844 staining MLH1 in wild-type Hap1 cells, with negative expression in MLH1 knockout Hap1 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised 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 ab223844 at 0.2 µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin at 0.5 µg/ml. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150119**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 647), pre-adsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.
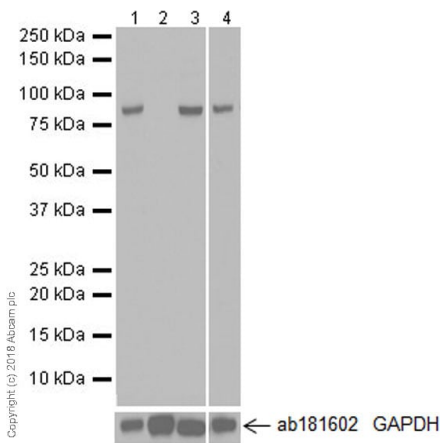
Western blot - Anti-MLH1 antibody [EPR20522] (ab223844)

All lanes : Anti-MLH1 antibody [EPR20522] (ab223844) at 1/1000 dilution

Lane 1 : SW480 (human colorectal adenocarcinoma cell line) whole cell lysate

Lane 2 : HCT 116 (human colorectal carcinoma epithelial cell line) whole cell lysate

Lane 3 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 4 : Human colon tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

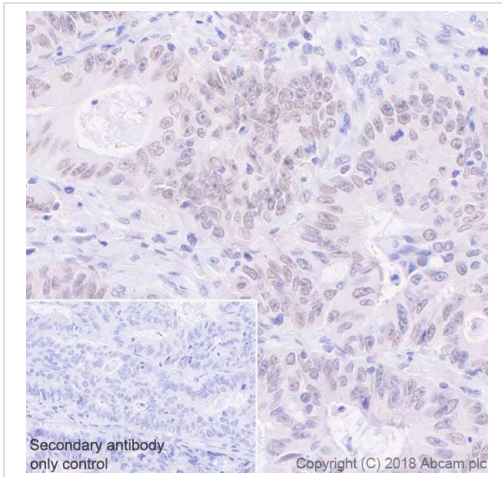**Predicted band size:** 84 kDa
Observed band size: 84 kDa

Exposure times.

Lanes 1-3: 8 seconds
Lane 4: 3 minutes.

The expression profile observed is consistent with the literature (PMID:15249596). The HCT116 cell line is a negative control for MLH1 (PMID: 23724141).
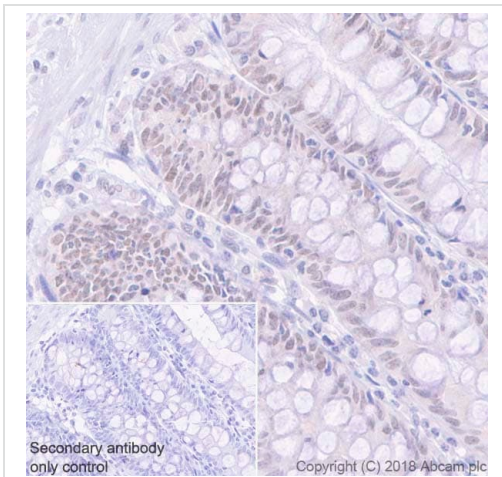
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR20522] (ab223844)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labelling MLH1 with ab223844 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining in tumor cells of human colon cancer is observed (PMID: 22608206). Counter stained with hematoxylin.

Secondary antobody only control: used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
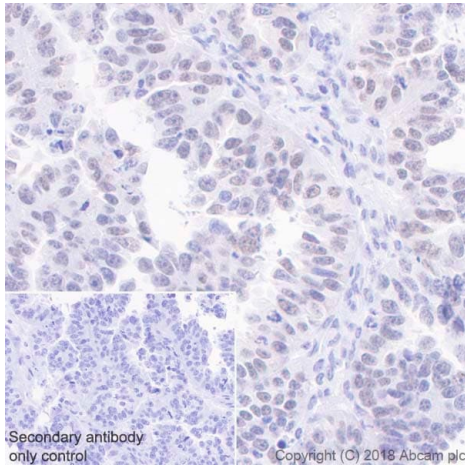

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR20522] (ab223844)

Immunohistochemical analysis of paraffin-embedded human colon tissue labelling MLH1 with ab223844 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining in human colon is observed (PMID: 10535979). Counter stained with hematoxylin.

Secondary antobody only control: used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLH1 antibody [EPR20522] (ab223844)

Immunohistochemical analysis of paraffin-embedded human ovary cancer tissue labelling MLH1 with ab223844 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining in tumor cells of human ovarian cancer (PMID: 10778972) is observed. Counter stained with hematoxylin.

Secondary antibody only control: used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
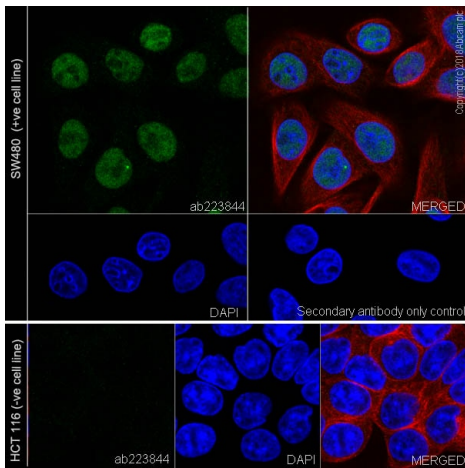

Immunocytochemistry/ Immunofluorescence - Anti-MLH1 antibody [EPR20522] (ab223844)

Immunofluorecent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilised SW480 (human colorectal adenocarcinoma cell line) cells labelling MLH1 with ab223844 at 1/100 dilution, followed by AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution (green). Confocal image showing nuclear staining in SW480 cell line.

DAPI was used as the Nuclear counterstain (blue). Alpha Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red).

The HCT 116 (human colorectal carcinoma epithelial) cell line is a negative control for MLH1 (PMID: 23724141).

Secondary antibody only control: PBS instead of ab223844, followed by AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution.
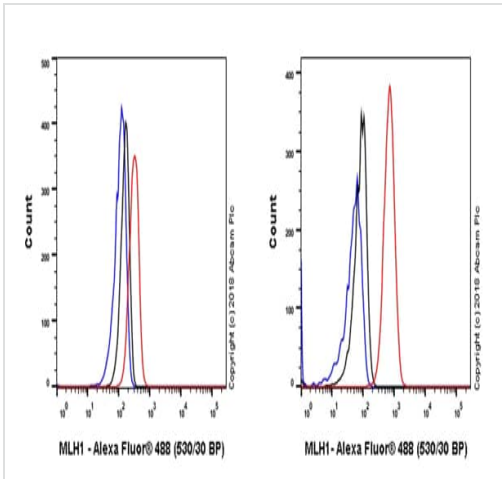
Flow Cytometry (Intracellular) - Anti-MLH1 antibody
[EPR20522] (ab223844)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HCT 116 (human colorectal carcinoma epithelial cell line, left) / SW480 (human colorectal adenocarcinoma cell line, right) cells labelling MLH1 with ab223844 at 1/600 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**)at 1/2000 dilution was used as the secondary antibody.

The HCT 116 (human colorectal carcinoma epithelial) cell line is a negative control for MLH1 (PMID: 23724141) (left panel).
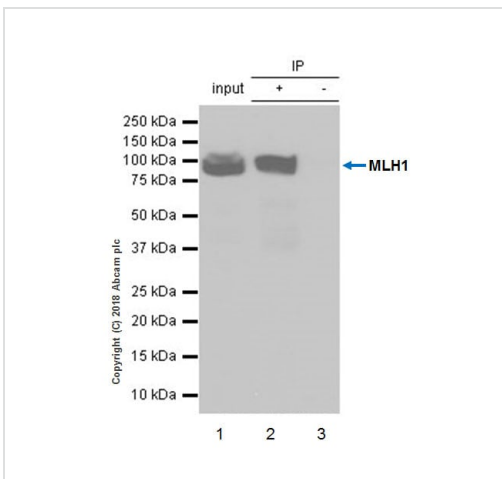
Immunoprecipitation - Anti-MLH1 antibody
[EPR20522] (ab223844)

MLH1 was immunoprecipitated from 0.35 mg HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab223844 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab223844 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/10000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (input).
Lane 2: ab223844 IP in HeLa whole cell lysate.
Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) instead of ab223844 in HeLa whole cell lysate.

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

Exposure time: Less than 1 second.

Why choose a
recombinant antibody?

Research with confidence
Consistent and reproducible results

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Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

Anti-MLH1 antibody [EPR20522] (ab223844)

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