

# Human MLH1 knockout HeLa cell line ab267223

6 Images

### Overview

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<b>Product name</b>	Human MLH1 knockout HeLa cell line
<b>Parental Cell Line</b>	HeLa
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, 10 bp deletion in exon 12 and 1 bp deletion in exon 12 and 4 bp deletion in exon 12
<b>Passage number</b>	<20
<b>Knockout validation</b>	Sanger Sequencing, Western Blot (WB)
<b>Tested applications</b>	<b>Suitable for:</b> WB
<b>Biosafety level</b>	2
<b>General notes</b>	<p><b>Recommended control:</b> Human wild-type HeLa cell line (<a href="#">ab255928</a>). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.</p> <p><b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p><b>Culture medium:</b> DMEM (High Glucose) + 10% FBS</p> <p><b>Initial handling guidelines:</b> Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability.</p> <ol style="list-style-type: none"><li>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li><li>2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.</li><li>3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of <math>2 \times 10^4</math> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li><li>4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li></ol> <p><b>Subculture guidelines:</b></p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of <math>2 \times 10^4</math> cells/cm<sup>2</sup> is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if</p>

required.

Cells should be passaged when they have achieved 80-90% confluence.

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We will provide viable cells that proliferate on revival.

## Properties

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<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Cervix
<b>Cell type</b>	epithelial
<b>Disease</b>	Adenocarcinoma
<b>Gender</b>	Female
<b>STR Analysis</b>	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10
<b>Antibiotic resistance</b>	Puromycin 1.00µg/ml
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

## Target

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<b>Function</b>	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.
<b>Tissue specificity</b>	Colon, lymphocytes, breast, lung, spleen, testis, prostate, thyroid, gall bladder and heart.
<b>Involvement in disease</b>	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.

**Applications**

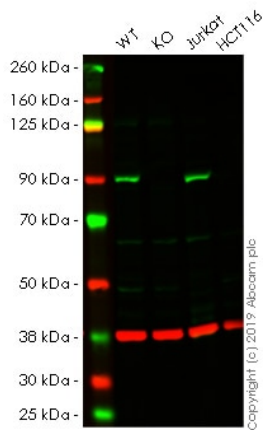
**The Abpromise guarantee**

Our **Abpromise guarantee** covers the use of ab267223 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		Use at an assay dependent concentration. Predicted molecular weight: 85 kDa.

**Images**



Western blot - Human MLH1 knockout HeLa cell line (ab267223)

**All lanes** : Anti-MLH1 antibody [EPR3893] ([ab108622](#)) at 1/1000 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.

### Secondary

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

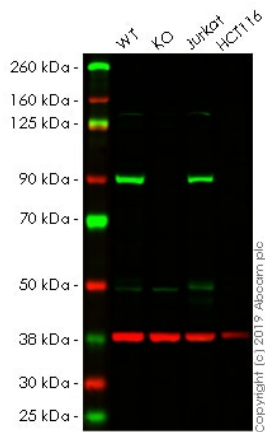
Performed under reducing conditions.

**Predicted band size:** 85 kDa

**Observed band size:** 85 kDa

**Lanes 1-4:** Merged signal (red and green). Green - [ab108622](#) observed at 90 kDa. Red - loading control [ab8245](#) observed at 37 kDa.

[ab108622](#) Anti-MLH1 antibody [EPR3893] 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. [ab108622](#) and Anti-GAPDH antibody [6C5] - Loading Control were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed ([ab216772](#)) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed ([ab216777](#)) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human MLH1 knockout HeLa cell line (ab267223)

**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.

### Secondary

**All lanes** : 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

**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.

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Mut  CTGTTCCCGGAATCTGTACGAACCATCTG-----ACCTTATCACTACTCCAGA
      |||
WT   CTGTTCCCGGAATCTGTACGAACCATCTGGTGGGCATAGACCTTATCACTACTCCAGA

```

Allele-1: 10 bp deletion in exon12

Sanger Sequencing - Human MLH1 knockout HeLa cell line (ab267223)

```

Mut  CTGTTCCCGGAATCTGTACGAACCATCTG---GCATAGACCTTATCACTACTCCAGA
      |||
WT   CTGTTCCCGGAATCTGTACGAACCATCTGGTGGGCATAGACCTTATCACTACTCCAGA

```

Allele-2: 4 bp deletion in exon 12.

Sanger Sequencing - Human MLH1 knockout HeLa cell line (ab267223)

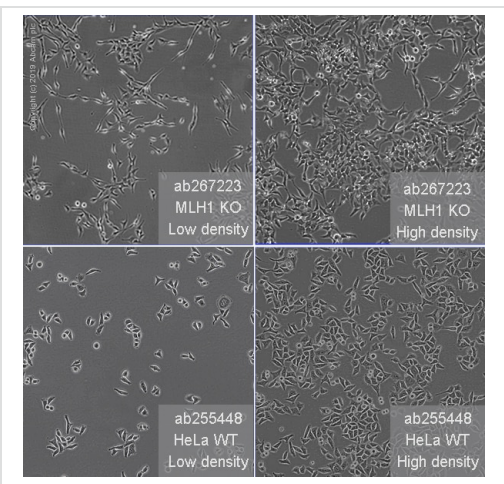
```

Mut  CTGTTCCCGGAATCTGTACGAACCATCTG-TGGGCATAGACCTTATCACTACTCCAGA
      |||
WT   CTGTTCCCGGAATCTGTACGAACCATCTGGTGGGCATAGACCTTATCACTACTCCAGA

```

Allele-3: 1 bp deletion in exon 12.

Sanger Sequencing - Human MLH1 knockout HeLa cell line (ab267223)



Representative images of MLH1 knockout HeLa cells, low and high confluency examples (top left and right respectively) and wild-type HeLa cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS XL Core microscope.

Cell Culture - Human MLH1 knockout HeLa cell line (ab267223)

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