

Human MLH1 knockout A549 cell line ab276105

3 图像

概述

产品名称	人MLH1 knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 70 bp deletion in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
经测试应用	适用于: WB
Biosafety level	1
常规说明	<p>Recommended control: Human wild-type A549 cell line (ab275463). 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>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: F-12K + 10% FBS</p> <p>Initial handling guidelines: 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"> 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 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. 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 2×10^3-1×10^4 cells/cm². Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 6×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

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

Do not exceed 7×10^4 cells/cm².

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We will provide viable cells that proliferate on revival.

性能

Number of cells	1 x 10 ⁶ cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
Mycoplasma free	Yes
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	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.
组织特异性	Colon, lymphocytes, breast, lung, spleen, testis, prostate, thyroid, gall bladder and heart.
疾病相关	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.

序列相似性

Belongs to the DNA mismatch repair mutL/hexB family.

细胞定位

Nucleus.

应用

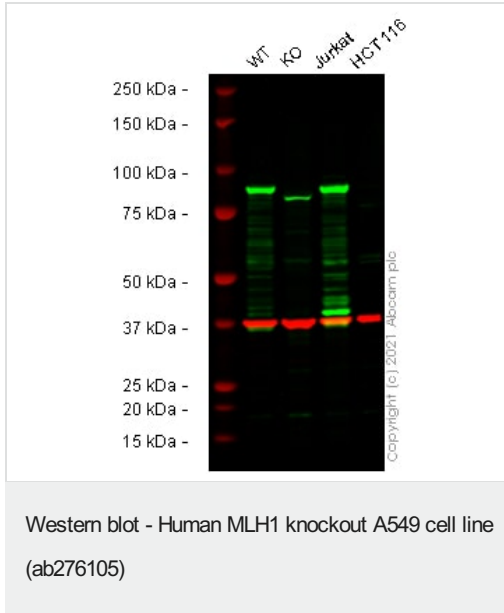
The Abpromise guarantee

Abpromise™ 承诺保证使用 ab276105 于以下的经测试应用

“应用说明”部分下显示的仅为推荐的起始稀释度；实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 85 kDa.

图片



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

Lane 1 : Wild-type A549 cell lysate

Lane 2 : MLH1 knockout A549 cell lysate

Lane 3 : Jurkat cell lysate

Lane 4 : HCT 116 cell lysate

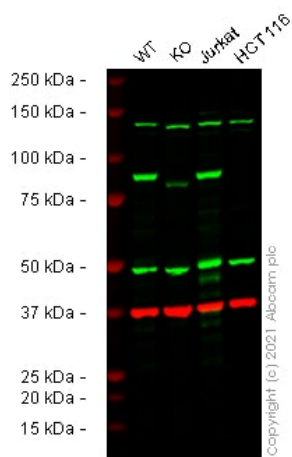
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 85 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 knockout cell line ab276105 (knockout cell lysate [ab288239](#)). The band observed in the knockout 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 knockout 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 - Human MLH1 knockout A549 cell line (ab276105)

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

Lane 1 : Wild-type A549 cell lysate

Lane 2 : MLH1 knockout 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: 85 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 knockout cell line ab276105 (knockout cell lysate [ab288239](#)). The band observed in the knockout 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 knockout 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.

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E4  AAAGAAGATCTGGATATTGTATGTG-----
    |||
WT  AAAGAAGATCTGGATATTGTATGTGAAAGSTTCACCTACTAGTAAACTGCAGTCTTTGAGGATTAGCCAGTATT

E4  -----TGAGGCTTTGGCCAGCATAAGCCATGTGGCTCATGTACTATTACAAAGAAAACA
    |||
WT  TCTAOCCTATGACCTTTCAGAGTGGAGCTTTGGCCAGCATAAGCCATGTGGCTCATGTACTATTACAAAGAAAACA

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70 bp deletion in exon 3

Sanger Sequencing - Human MLH1 knockout A549 cell line (ab276105)

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