abcam

Product datasheet

Human MCL1 knockout HEK-293T cell line ab266838

3 图像

概述

常规说明

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 1 and 7 bp insertion in exon 1

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

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经测试应用 适用于: WB

Biosafety level

-

Recommended control: Human wild-type HEK293T cell line (<u>ab255449</u>). 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.

Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.

Culture medium: DMEM (High Glucose) + 10% FBS

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.

- 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 2x10⁴ 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.

Subculture guidelines:

All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm² is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

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

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We will provide viable cells that proliferate on revival.

性能

Number of cells 1 x 10⁶ cells/vial, 1 mL

Adherent / Suspension Adherent
Tissue Kidney

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01:

7, 9.3 TPOX: 11 CSF1PO: 11, 12

Antibiotic resistance Puromycin 1.00μg/ml

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 Involved in the regulation of apoptosis versus cell survival, and in the maintenance of viability but

not of proliferation. Mediates its effects by interactions with a number of other regulators of

apoptosis. Isoform 1 inhibits apoptosis. Isoform 2 promotes apoptosis.

序列相似性 Belongs to the Bcl-2 family.

翻译后修饰 Cleaved by CASP3 during apoptosis. In intact cells cleavage occurs preferentially after Asp-127,

yielding a pro-apoptotic 28 kDa C-terminal fragment.

Rapidly degraded in the absence of phosphorylation on Thr-163 in the PEST region.

Phosphorylated on Thr-163. Treatment with taxol or okadaic acid induces phosphorylation on

additional sites.

细胞定位 Membrane. Cytoplasm. Mitochondrion. Nucleus > nucleoplasm. Cytoplasmic, associated with

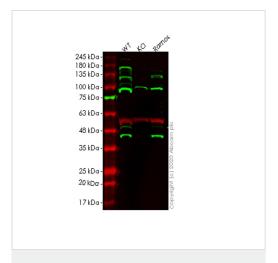
mitochondria.

应用

The Abpromise guarantee Abpromise™承诺保证使用ab266838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: 37 kDa.



Western blot - Human MCL1 knockout HEK293T cell line (ab266838)

All lanes: Anti-MCL1 antibody [Y37] (ab32087) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: MCL1 knockout HEK293T cell lysate

Lane 3: Ramos cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Predicted band size: 37 kDa **Observed band size:** 37 kDa

Lanes 1-3: Merged signal (red and green). Green - <u>ab32087</u> observed at 37 kDa. Red - loading control <u>ab7291</u> observed at 50 kDa.

ab32087 Anti-MCL1 antibody [Y37] was shown to specifically react with MCL1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266838 (knockout cell lysate ab256986) was used. Wild-type and MCL1 knockout samples were subjected to SDS-PAGE. ab32087 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Mut GGCGACTGGCAATGTTTGGCCTCAAAAGAA-CGCGGTAATCGGACTCAACCTCTACTGTG

WT GGCGACTGGCAATGTTTGGCCTCAAAAGAA-CGCGGTAATCGGACTCAACCTCTACTGTG

Sanger Sequencing - Human MCL1 knockout HEK293T cell line (ab266838)

Allele-1: 1 bp deletion in exon 1

Allele-2: 7 bp insertion in exon 1.

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