abcam

Product datasheet

Human MYC (c-Myc) knockout HEK-293T cell line ab256500

★★★★★ 1 Abreviews 6 图像

概述

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous (4N): 1bp Tinsertion (2N); 8 bp

deletion and C to Tinsertion (1N); 4 bp deletion in exon 2 (1N)

Passage number <2

Knockout validation Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)

经测试应用 适用于: ICC, WB

Biosafety level 2

常规说明

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% CO_2 . 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

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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.

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

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 Participates in the regulation of gene transcription. Binds DNA in a non-specific manner, yet also

specifically recognizes the core sequence 5'-CAC[GA]TG-3'. Seems to activate the transcription

of growth-related genes.

疾病相关 Note=Overexpression of MYC is implicated in the etiology of a variety of hematopoietic tumors.

Note=A chromosomal aberration involving MYC may be a cause of a form of B-cell chronic

lymphocytic leukemia. Translocation t(8;12)(q24;q22) with BTG1.

Defects in MYC are a cause of Burkitt lymphoma (BL) [MIM:113970]. A form of undifferentiated malignant lymphoma commonly manifested as a large osteolytic lesion in the jaw or as an abdominal mass. Note=Chromosomal aberrations involving MYC are usually found in Burkitt

lymphoma. Translocations t(8;14), t(8;22) or t(2;8) which juxtapose MYC to one of the heavy or

light chain immunoglobulin gene loci.

序列相似性 Contains 1 basic helix-loop-helix (bHLH) domain.

翻译后修饰 Phosphorylated by PRKDC. Phosphorylation at Thr-58 and Ser-62 by GSK3 is required for

ubiquitination and degradation by the proteasome.

Ubiquitinated by the SCF(FBXW7) complex when phosphorylated at Thr-58 and Ser-62, leading to its degradation by the proteasome. In the nucleoplasm, ubiquitination is counteracted by USP28, which interacts with isoform 1 of FBXW7 (FBW7alpha), leading to its deubiquitination and preventing degradation. In the nucleolus, however, ubiquitination is not counteracted by

USP28, due to the lack of interaction between isoform 4 of FBXW7 (FBW7gamma) and USP28, explaining the selective MYC degradation in the nucleolus. Also polyubiquitinated by the

DCX(TRUSS) complex.

细胞定位 Nucleus > nucleoplasm. Nucleus > nucleolus.

形式 c-Myc is also expressed in the cytoplasm.

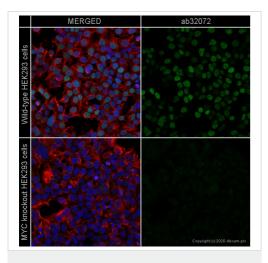
The Abpromise guarantee

Abpromise™承诺保证使用ab256500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。

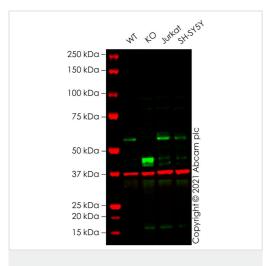
应用	Ab评论	说明
ICC		Use at an assay dependent concentration.
WB	****(1)	Use at an assay dependent concentration. Predicted molecular weight: 48 kDa.

图片



Immunocytochemistry/ Immunofluorescence -Human MYC (c-Myc) knockout HEK-293T cell line (ab256500) **ab32072** staining MYC in wild-type HEK293 cells (top panel) and MYC knockout HEK293 cells (ab256500) (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized 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 with **ab32072** at 5µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit lgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse lgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Human MYC (c-Myc) knockout HEK-293T cell line (ab256500) **All lanes :** Anti-Myc tag antibody [Myc.A7] (<u>ab18185</u>) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: MYC knockout HEK-293T cell lysate

Lane 3 : Jurkat cell lysate

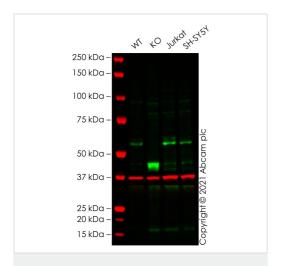
Lane 4: SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa

False colour image of Western blot: Anti-Myc tag antibody [Myc.A7] staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab18185 was shown to bind specifically to Myc tag. A band was observed at 57 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in MYC knockout cell line ab256500 (knockout cell lysate ab263850). The band observed in the knockout lysate lane below 57 kDa is likely to represent a truncated form of Myc tag. 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 MYC knockout HEK-293T 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit lgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.



Western blot - Human MYC (c-Myc) knockout HEK-293T cell line (ab256500)

All lanes: Anti-Myc tag antibody [9E10] (ab32) at 1/200 dilution

Lane 2: MYC knockout HEK-293T cell lysate

Lane 3 : Jurkat cell lysate

Lane 4 : SH-SY5Y cell lysate

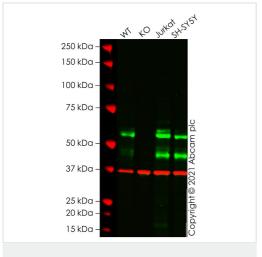
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa

False colour image of Western blot: Anti-Myc tag antibody [9E10] staining at 1/200 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32 was shown to bind specifically to Myc tag. A band was observed at 57 kDa in wild-type HEK-293T cell lysates with no signal observed at this size

in MYC knockout cell line ab256500 (knockout cell lysate ab263850). The band observed in the knockout lysate lane below 57 kDa is likely to represent a truncated form of Myc tag. 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 MYC knockout HEK-293T 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit lgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.



Western blot - Human MYC (c-Myc) knockout HEK-293T cell line (ab256500)

All lanes : Anti-c-Myc antibody [Y69] - ChIP Grade (<u>ab32072</u>) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: MYC knockout HEK-293T cell lysate

Lane 3 : Jurkat cell lysate

Lane 4 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa

False colour image of Western blot: Anti-c-Myc antibody [Y69] 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, ab32072 was shown to bind specifically to c-Myc. A band was observed at 45/57 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in MYC knockout cell line ab256500 (knockout cell lysate ab263850). The band observed in the knockout lysate lane below 45/57 kDa is likely to represent a truncated form of c-Myc. 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 MYC knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % 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 lgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.

260 kDa - 160 kDa - 125 kDa - 90 kDa - 70 kDa - 38 kDa - 30 kDa - 35 kDa - 35 kDa - 15 kDa -

Western blot - Human MYC knockout HEK293T cell line (ab256500)

All lanes : Anti-c-Myc antibody [Y69] - ChIP Grade (**ab32072**) at 1/1000 dilution

Lane 1 : Jurkat cell lysate

Lane 2 : HeLa cell lysate

Lane 3: Wild-type HEK-293T cell lysate

Lane 4: MYC knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 48 kDa

Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab32072</u> observed at 57 kDa. Red - loading control, <u>ab8245</u> observed at 37 kDa.

ab32072 was shown to react with MYC in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab256500 (knockout cell lysate ab263850) was used. Wild-type and MYC knockout samples were subjected to SDS-PAGE. ab32072 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 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.

Mut TCACCAACAGGAATCTATGACCTCGACTACGACTCGGTGCAGCCGTATTTCTACTGCGAC

WT TCACCAACAGGAA CTATGACCTCGACTACGACTCGGTGCAGCCGTATTTCTACTGCGAC

Sanger Sequencing - Human MYC knockout

HEK293T cell line (ab256500)

Homozygous: 1 bp insertion in exon 2

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