abcam

Product datasheet

Human CA2 (Carbonic anhydrase 2) knockout HEK-293T cell line ab265072

3 图像

概述

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

2

经测试应用 适用于: WB

Biosafety level

常规说明

Recommended control: Human wild-type HEK293T cell line (<u>ab255593</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.

1

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if 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: 11, 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 15, 20

TH01: 7, 9.3 TPOX: 11, 12 CSF1PO: 12

Antibiotic resistance Puromycin 1.00 µg/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 Essential for bone resorption and osteoclast differentiation (By similarity). Reversible hydration of

carbon dioxide. Can hydrates cyanamide to urea. Involved in the regulation of fluid secretion into

the anterior chamber of the eye.

疾病相关 Defects in CA2 are the cause of osteopetrosis autosomal recessive type 3 (OPTB3)

[MIM:259730]; also known as osteopetrosis with renal tubular acidosis, carbonic anhydrase II deficiency syndrome, Guibaud-Vainsel syndrome or marble brain disease. Osteopetrosis is a rare genetic disease characterized by abnormally dense bone, due to defective resorption of immature bone. The disorder occurs in two forms: a severe autosomal recessive form occurring in utero, infancy, or childhood, and a benign autosomal dominant form occurring in adolescence or adulthood. Autosomal recessive osteopetrosis is usually associated with normal or elevated amount of non-functional osteoclasts. OPTB3 is associated with renal tubular acidosis, cerebral

calcification (marble brain disease) and in some cases with mental retardation.

序列相似性 Belongs to the alpha-carbonic anhydrase family.

细胞定位 Cytoplasm.

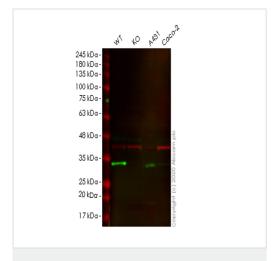
应用

The Abpromise guarantee Abpromise™承诺保证使用ab265072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: 29 kDa.

图片



Western blot - Human CA2 knockout HEK293T cell line (ab265072)

All lanes : Anti-Carbonic anhydrase 2/CA2 antibody (<u>ab82559</u>) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: CA2 knockout HEK293T cell lysate

Lane 3 : A431 cell lysate
Lane 4 : Caco-2 cell lysate

Lysates/proteins at 20 µg per lane.

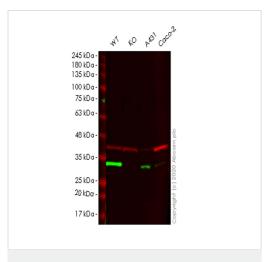
Secondary

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

Predicted band size: 29 kDa
Observed band size: 29 kDa

Lanes 1-4: Merged signal (red and green). Green - <u>ab82559</u> observed at 29 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab82559 Anti-Carbonic anhydrase 2/CA2 antibody was shown to specifically react with Carbonic anhydrase 2/CA2 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab265072 (knockout cell lysate ab257084) was used. Wild-type and Carbonic anhydrase 2/CA2 knockout samples were subjected to SDS-PAGE. ab82559 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2. 5 hours 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.



Western blot - Human CA2 knockout HEK293T cell line (ab265072)

All lanes : Anti-Carbonic anhydrase 2/CA2 antibody [EPR5195] (ab124687) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: CA2 knockout HEK293T cell lysate

Lane 3 : A431 cell lysate
Lane 4 : Caco-2 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 29 kDa Observed band size: 29 kDa

Lanes 1-4: Merged signal (red and green). Green - <u>ab124687</u> observed at 29 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab124687 Anti-Carbonic anhydrase 2/CA2 antibody [EPR5195] was shown to specifically react with Carbonic anhydrase 2/CA2 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab265072 (knockout cell lysate ab257084) was used. Wild-type and Carbonic anhydrase 2/CA2 knockout samples were subjected to SDS-PAGE. ab124687 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2.5 hours 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 GCGCTGGCCGTCGCCGGCACTCACCGTTGTTGTTTGCCGTACCCCCAGTGATGGGACATG

WT GCGCTGGCCGTCGCCGGCACTCACCGTTGT GTTTGCCGTACCCCCAGTGATGGGACATG

Sanger Sequencing - Human CA2 knockout HEK293T cell line (ab265072)

Homozygous: 1 bp insertion in exon 1.

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