abcam

Product datasheet

Human CA2 (Carbonic anhydrase 2) knockout HEK-293T cell lysate ab257084

3 Images

Overview

Product name Human CA2 (Carbonic anhydrase 2) knockout HEK-293T cell lysate

Product overview

Knockout cell lysate achieved by CRISPR/Cas9.

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)

Reconstitution notesTo use as WB control, resuspend the lyophilizate in 50 μL of LDS* Sample Buffer to have a final

concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M

DTT.

*Usage of SDS sample buffer is not recommended with these lyophilized lysates.

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease

inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are

prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -

20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

See here for more information on knockout cell lysates.

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products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH

Authorisation, and any other relevant authorisations, for their intended uses.

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licenses and patents please refer to our <u>limited use license</u> and <u>patent pages</u>.

Tested applications Suitable for: WB

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Properties

Storage instructions

Store at -80°C. Please refer to protocols.

Components	1 kit
ab263472 - Human CA2 knockout HEK293T cell lysate	1 x 100μg
ab255594 - Human wild-type HEK293T cell lysate	1 x 100µg

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

Target

Function

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.

Involvement in disease

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.

Sequence similarities

Belongs to the alpha-carbonic anhydrase family.

Cellular localization

Cytoplasm.

Applications

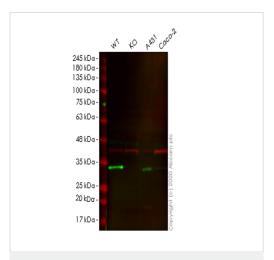
The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab257084 in the following tested applications.

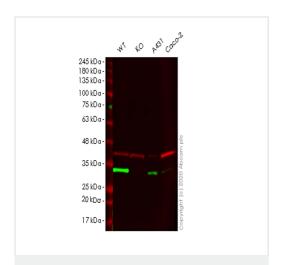
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 29 kDa.

Images



Western blot - Human CA2 knockout HEK293T cell lysate (ab257084)



Western blot - Human CA2 knockout HEK293T cell lysate (ab257084)

Lane 1: Wild-type HEK293T cell lysate (20 ug)

Lane 2:CA2 knockout HEK293T cell lysate (20 ug)

Lane 3:A431 cell lysate (20 ug)

Lane 4: Caco-2 cell lysate (20 ug)

ab82559 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.

Lane 1: Wild-type HEK293T cell lysate (20 ug)

Lane 2:CA2 knockout HEK293T cell lysate (20 ug)

Lane 3:A431 cell lysate (20 ug)

Lane 4: Caco-2 cell lysate (20 ug)

ab124687 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 lysate (ab257084) Homozygous: 1 bp insertion in exon 1

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