abcam

Product datasheet

Human MCAM (CD146) knockout HeLa cell lysate ab256985

2 图像

概述

产品概述 Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the

protein of interest. Please see data images.

Parental Cell Line HeLa

Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 19 bp deletion in exon 6.

Passage number <20

Knockout validation Sanger Sequencing

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.

说明

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.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of

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

It is the responsibility of our customers to check the necessity of application of $\ensuremath{\mathsf{REACH}}$

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

This product is subject to limited use licenses from The Broad Institute and ERS Genomics

Limited, and is developed with patented technology. For full details of the limited use licenses and

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

经测试应用 适用于: WB

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存放说明

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

组 件	1 kit
ab260884 - Human MCAM knockout HeLa cell lysate	1 x 100μg
ab255929 - Human wild-type HeLa cell lysate	1 x 100μg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18

TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10

靶标

功能

Plays a role in cell adhesion, and in cohesion of the endothelial monolayer at intercellular junctions in vascular tissue. Its expression may allow melanoma cells to interact with cellular elements of the vascular system, thereby enhancing hematogeneous tumor spread. Could be an adhesion molecule active in neural crest cells during embryonic development. Acts as surface receptor that triggers tyrosine phosphorylation of FYN and PTK2, and a transient increase in the intracellular calcium concentration.

组织特异性

Detected in endothelial cells in vascular tissue throughout the body. May appear at the surface of neural crest cells during their embryonic migration. Appears to be limited to vascular smooth muscle in normal adult tissues. Associated with tumor progression and the development of metastasis in human malignant melanoma. Expressed most strongly on metastatic lesions and advanced primary tumors and is only rarely detected in benign melanocytic nevi and thin primary melanomas with a low probability of metastasis.

序列相似性

Contains 3 lg-like C2-type (immunoglobulin-like) domains. Contains 2 lg-like V-type (immunoglobulin-like) domains.

细胞定位

Membrane.

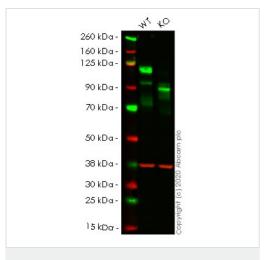
应用

The Abpromise guarantee

Abpromise™承诺保证使用ab256985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: 72 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.



Western blot - Human MCAM (CD146) knockout HeLa cell lysate (ab256985)

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: MCAM knockout HeLa cell lysate (20µg)

Lanes 1-2: Merged signal (red and green). Green - <u>ab75769</u> observed at 120 kDa. Red - loading control, <u>ab8245</u> observed at 37 kDa.

ab75769 Anti-CD146 antibody [EPR3208] was shown to specifically react with CD146 in wild-type HeLa cells in western blot. The band observed in the knockout cell line ab261790 (knockout cell lysate ab256985) lane below 120kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and CD146 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab75769 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 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	CATGAAGGAGTTGTTTTCTGTGAGTACTAACCTGACTCTCT			
WT	CATGAAGGAGTCCAGGGAAGTCACCGTCCCTGTTTTCTGTGAGTACTAACCTGACTCTCT			
80	anger Sequencing - Human MCAM knockout HeLa			
Sanger Sequencing - Human McAW Knockout HeLa				
cell lysate (ab256985)				

Homozygous: 19 bp deletion in exon 6

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