abcam

Product datasheet

Human MCAM (CD146) knockout HeLa cell lysate ab256985

2 Images

Overview

Product name	Human MCAM (CD146) knockout HeLa cell lysate
Product overview	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 notes	To 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.** 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 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 **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

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

Components	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

Target

Function 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.

Tissue specificity 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.

Sequence similarities Contains 3 Ig-like C2-type (immunoglobulin-like) domains.
Contains 2 Ig-like V-type (immunoglobulin-like) domains.

Cellular localization Membrane.

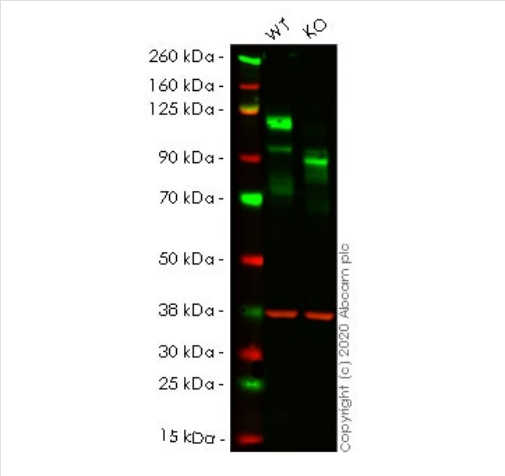
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256985 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: 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.

Images



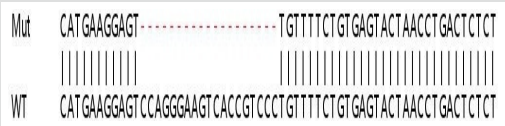
Western blot - Human MCAM (CD146) knockout HeLa cell lysate (ab256985)

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 - **ab75769** observed at 120 kDa. Red - loading control, **ab8245** 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 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.



Sanger Sequencing - Human MCAM knockout HeLa cell lysate (ab256985)

Homozygous: 19 bp deletion in exon 6

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