# abcam

# Product datasheet

# Human MCAM (CD146) knockout HeLa cell lysate ab256985

### 画像数 2

#### 製品の概要

製品名 Human MCAM (CD146) knockout HeLa cell lysate

製品の概要 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.

 $\hbox{$^*$Usage of SDS sample buffer is not recommended with these lyophilized lysates.}$ 

特記事項

**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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**アプリケーション 適用あり**: WB

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#### 製品の特性

#### 保存方法

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

| 内容  | 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

#### ターゲット情報

機能

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.

組織特異性

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.

配列類似性

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

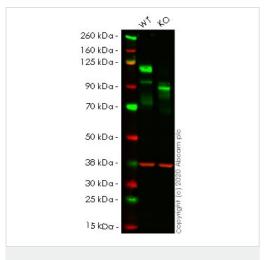
細胞内局在

Membrane.

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab256985の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション | Abreviews | 特記事項  |
|----------|-----------|---|
| 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  | CAT GAAGGAGT        | TGTTTTCTGTGAGTACTAACCTGACTCTCT                       |  |  |
|--|---------------------|--|--|--|
|  |                     |  |  |  |
| WT   | CATGAAGGAGTCCAGGGAA | GT CACCGT CCCT GTTTT CT GT GAGT ACT AACCT GACT CT CT |  |  |
| _  |                     |  |  |  |
| Sanger Sequencing - Human MCAM knockout HeLa |                     |  |  |  |
| cell lysate (ab256985)                       |                     |  |  |  |
|  |                     |  |  |  |

Homozygous: 19 bp deletion in exon 6

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