

Human CARM1 knockout HEK-293T cell line ab266557

3 Images

Overview

Product name	Human CARM1 knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Recommended control: Human wild-type HEK293T cell line (ab255449). 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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 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. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if</p>

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.

Properties

Number of cells	1 x 10 ⁶ cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Methylates (mono- and asymmetric dimethylation) the guanidino nitrogens of arginyl residues in several proteins involved in DNA packaging, transcription regulation, pre-mRNA splicing, and mRNA stability. Recruited to promoters upon gene activation together with histone acetyltransferases from EP300/P300 and p160 families, methylates histone H3 at 'Arg-17' (H3R17me), forming mainly asymmetric dimethylarginine (H3R17me ₂), leading to activate transcription via chromatin remodeling. During nuclear hormone receptor activation and TCF7L2/TCF4 activation, acts synergically with EP300/P300 and either one of the p160 histone acetyltransferases NCOA1/SRC1, NCOA2/GRIP1 and NCOA3/ACTR or CTNNB1/beta-catenin to activate transcription. During myogenic transcriptional activation, acts together with NCOA3/ACTR as a coactivator for MEF2C. During monocyte inflammatory stimulation, acts together with EP300/P300 as a coactivator for NF-kappa-B. Acts as coactivator for PPARG, promotes adipocyte differentiation and the accumulation of brown fat tissue. Plays a role in the regulation of pre-mRNA alternative splicing by methylation of splicing factors. Also seems to be involved in p53/TP53 transcriptional activation. Methylates EP300/P300, both at 'Arg-2142', which may loosen its interaction with NCOA2/GRIP1, and at 'Arg-580' and 'Arg-604' in the KIX domain, which impairs its interaction with CREB and inhibits CREB-dependent transcriptional activation. Also methylates arginine residues in RNA-binding proteins PABPC1, ELAVL1 and ELAV4, which may affect their mRNA-stabilizing properties and the half-life of their target mRNAs.
Tissue specificity	Overexpressed in prostate adenocarcinomas and high-grade prostatic intraepithelial neoplasia.
Sequence similarities	Belongs to the protein arginine N-methyltransferase family.
Post-translational modifications	Auto-methylated on Arg-550. Methylation enhances transcription coactivator activity. Methylation is required for its role in the regulation of pre-mRNA alternative splicing. Phosphorylation at Ser-216 interferes with S-adenosyl-L-methionine binding and strongly reduces methyltransferase activity (By similarity). Phosphorylation at Ser-216 is strongly increased during

mitosis, and decreases rapidly to a very low, basal level after entry into the G1 phase of the cell cycle. Phosphorylation at Ser-216 may promote location in the cytosol.

Cellular localization

Nucleus. Cytoplasm. Mainly nuclear during the G1, S and G2 phases of the cell cycle. Cytoplasmic during mitosis, after breakup of the nuclear membrane.

Applications

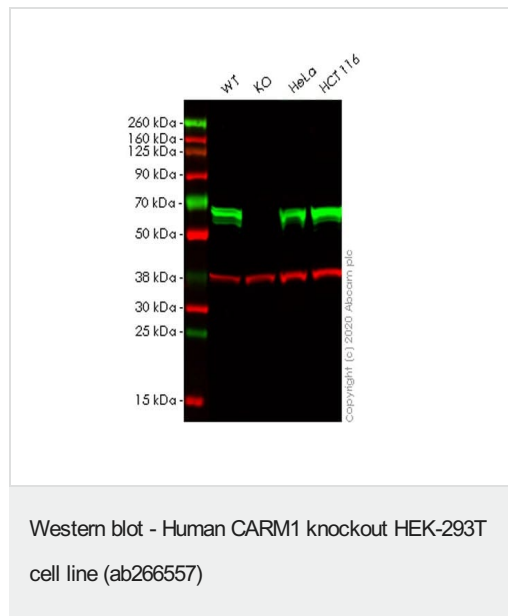
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab266557 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: 65 kDa.

Images



All lanes : Anti-CARM1 antibody [EPR23678-113] (**ab243638**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (human embryonic kidney epithelial cell), whole cell lysate

Lane 2 : CARM1 knockout HEK-293T (human embryonic kidney epithelial cell), whole cell lysate

Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lane 4 : HCT116 (human colorectal carcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) at 1/10000 dilution

Predicted band size: 65 kDa

Observed band size: 65 kDa

Blocking and diluting buffer and concentration: Intercept® (TBS)

Blocking Buffer diluted with an equal volume of 0.1% TBS.

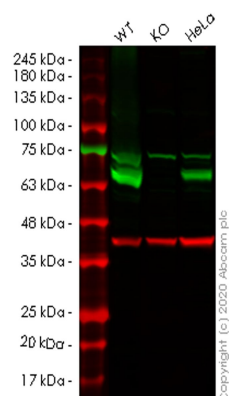
Lanes 1 - 4: Merged signal (red and green). Green - **ab243638** observed at 65 kDa. Red - loading control **ab8245** (Mouse

monoclonal [6C5] to GAPDH) observed at 36 kDa.

ab243638 was shown to react with CARM1 in wild-type HEK-293T cells in western blot with loss of signal observed in CARM1 knockout cell line ab266557 (CARM1 knockout cell lysate **ab257871**). Wild-type and CARM1 knockout HEK-293T cell lysates were subjected to SDS-PAGE.

ab243638 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4°C overnight at 1 in 1000 dilution 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 10000 dilution for 1 hour at room temperature before imaging.

Boiled samples are used in this blot.



Western blot - Human CARM1 knockout HEK293T cell line (ab266557)

All lanes : Anti-CARM1 antibody (**ab128851**) at 1/500 dilution

Lane 1 : Wild-type HEK293T cell lysate

Lane 2 : CARM1 knockout HEK293T cell lysate

Lane 3 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

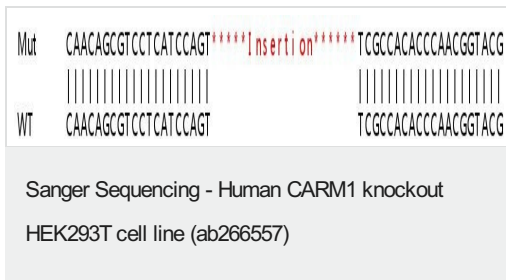
Predicted band size: 65 kDa

Observed band size: 66 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab128851** observed at 66 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab128851 Anti-CARM1 antibody was shown to specifically react with CARM1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266557 (knockout cell lysate **ab257871**) was used. Wild-type and CARM1 knockout samples were subjected to SDS-PAGE. **ab128851** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 500 dilution 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.



Homozygous: Insertion of the selection cassette in exon2

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