abcam

Product datasheet

# Human ACADVL (VLCAD) knockout HEK-293T cell line ab266484

3 Images

## Overview

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| **Product name** | Human ACADVL (VLCAD) 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 1 |
| **Passage number** | <20 |
| **Knockout validation** | Sanger Sequencing, Western Blot (WB) |
| **Tested applications** | **Suitable for:** WB |
| **Biosafety level** | 2 |
| **General notes** | **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.<br><br>**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.<br><br>**Culture medium:** DMEM (High Glucose) + 10% FBS<br><br>**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.<br><br>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.<br>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.<br>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 $2x10^4$ cells/$cm^2$. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.<br>4. Incubate the culture at 37°C incubator with 5% $CO_2$. Cultures should be monitored daily.<br><br>**Subculture guidelines:**<br><br>All seeding densities should be based on cell counts gained by established methods. |

A guide seeding density of $2x10^4$ cells/$cm^2$ is recommended.
A partial media change 24 hours prior to subculture may be helpful to encourage growth, if 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

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| **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

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| **Function** | Active toward esters of long-chain and very long chain fatty acids such as palmitoyl-CoA, mysritoyl-CoA and stearoyl-CoA. Can accomodate substrate acyl chain lengths as long as 24 carbons, but shows little activity for substrates of less than 12 carbons. |
| **Pathway** | Lipid metabolism; mitochondrial fatty acid beta-oxidation. |
| **Involvement in disease** | Defects in ACADVL are the cause of acyl-CoA dehydrogenase very long chain deficiency (ACADVLD) [MIM:201475]. ACADVLD is an autosomal recessive disease which leads to impaired long-chain fatty acid beta-oxidation. It is clinically heterogeneous, with three major phenotypes: a severe childhood form, with early onset, high mortality, and high incidence of cardiomyopathy; a milder childhood form, with later onset, usually with hypoketotic hypoglycemia as the main presenting feature, low mortality, and rare cardiomyopathy; and an adult form, with isolated skeletal muscle involvement, rhabdomyolysis, and myoglobinuria, usually triggered by exercise or fasting. |
| **Sequence similarities** | Belongs to the acyl-CoA dehydrogenase family. |
| **Cellular localization** | Mitochondrion inner membrane. |

## Applications

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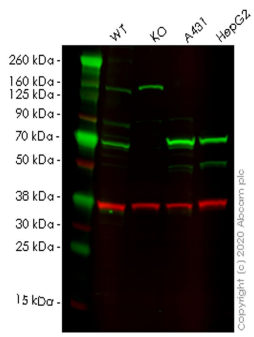
| **The Abpromise guarantee** | Our **Abpromise guarantee** covers the use of ab266484 in the following tested applications. |

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: 70 kDa. |

## Images




Western blot - Human ACADVL (VLCAD) knockout HEK293T cell line (ab266484)

**All lanes :** Anti-ACADVL/VLCAD antibody [EPR15107(B)] (**ab188872**) at 1/1000 dilution

**Lane 1 :** Wild-type HEK293T cell lysate
**Lane 2 :** ACADVL knockout HEK293T cell lysate
**Lane 3 :** A431 cell lysate
**Lane 4 :** HepG2 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

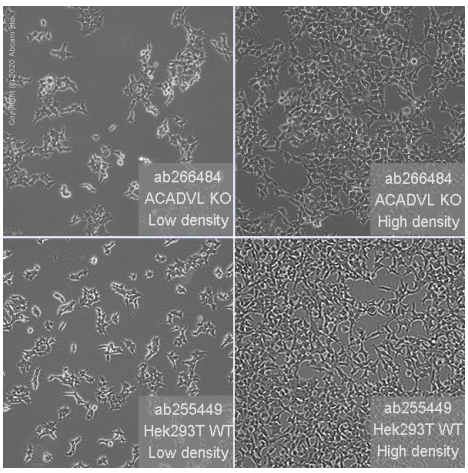
**Predicted band size:** 70 kDa
**Observed band size:** 70 kDa

**Lanes 1-4:** Merged signal (red and green). Green - **ab188872** observed at 70 kDa. Red - loading control **ab8245** observed at 36 kDa.

**ab188872** Anti-ACADVL/VLCAD antibody [EPR15107(B)] was shown to specifically react with ACADVL/VLCAD in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266484 (knockout cell lysate **ab257332**) was used. Wild-type and ACADVL/VLCAD knockout samples were subjected to SDS-PAGE. **ab188872** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C 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 20000 dilution for 1 hour at room temperature before imaging.


Sanger Sequencing - Human ACADVL knockout HEK293T cell line (ab266484)

Homozygous: Insertion of the selection cassette in exon 1




Cell Culture - Human ACADVL (VLCAD) knockout HEK293T cell line (ab266484)

Representative images of ACADVL knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.



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