abcam

Product datasheet

Human LMAN1 knockout HEK-293T cell line ab266248

5 Images

Overview

Product name	Human LMAN1 knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion 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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **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. 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 $2x10^4$ cells/cm^2. 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% CO_2. Cultures should be monitored daily. **Subculture guidelines:** 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.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

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
Antibiotic resistance	Puromycin 1.00µg/ml
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	Mannose-specific lectin. May recognize sugar residues of glycoproteins, glycolipids, or glycosylphosphatidyl inositol anchors and may be involved in the sorting or recycling of proteins, lipids, or both. The LMAN1-MCFD2 complex forms a specific cargo receptor for the ER-to-Golgi transport of selected proteins.
Tissue specificity	Ubiquitous.
Involvement in disease	Defects in LMAN1 are THE cause of factor V and factor VIII combined deficiency type 1 (F5F8D1) [MIM:227300]; also known as multiple coagulation factor deficiency I (MCFD1). F5F8D1 is an autosomal recessive blood coagulation disorder characterized by bleeding symptoms similar to those in hemophilia or parahemophilia, that are caused by single deficiency of FV or FVIII, respectively. The most common symptoms are epistaxis, menorrhagia, and excessive bleeding during or after trauma. Plasma levels of coagulation factors V and VIII are in the range of 5 to 30% of normal.
Sequence similarities	Contains 1 L-type lectin-like domain.
Post-translational modifications	The N-terminal may be partly blocked.
Cellular localization	Endoplasmic reticulum-Golgi intermediate compartment membrane. Golgi apparatus membrane. Endoplasmic reticulum membrane.

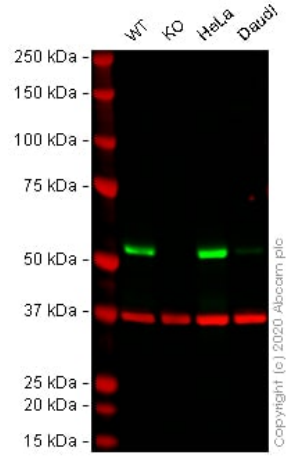
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266248 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: 58 kDa.

Images



Western blot - Human LMAN1 knockout HEK293T cell line (ab266248)

All lanes : Anti-LMAN1 antibody [OTI1B8] (**ab118407**) at 1/200 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : LMAN1 knockout HEK-293T cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Daudi cell lysate

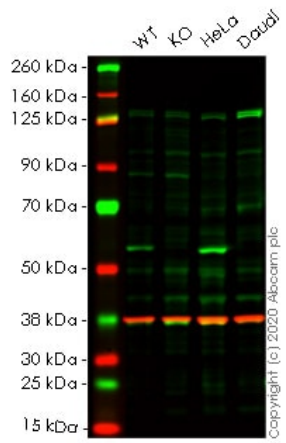
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 55 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab118407** observed at 55 kDa. Red - loading control **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37kDa.

ab118407 was shown to react with LMAN1 in wild-type HEK-293T cells in western blot with loss of signal observed in LMAN1 knockout cell line ab266248 (LMAN1 knockout cell lysate **ab257505**). Wild-type and LMAN1 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab118407** and **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4°C at a 1 in 200 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human LMAN1 knockout HEK293T cell line (ab266248)

All lanes : Anti-LMAN1 antibody [EPR6980] (**ab126720**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : LMAN1 knockout HEK-293T cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Daudi cell lysate

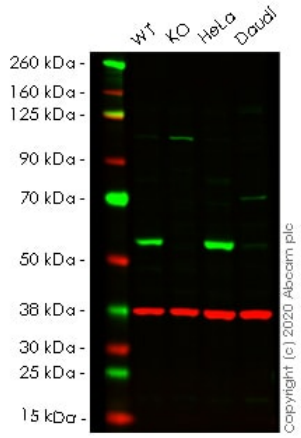
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 55 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab126720** observed at 55 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab126720 Anti-LMAN1 antibody [EPR6980] was shown to specifically react with Protein ERGIC-53 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266248 (knockout cell lysate **ab257505**) was used. Wild-type and Protein ERGIC-53 knockout samples were subjected to SDS-PAGE. **ab126720** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°CC 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.



Western blot - Human LMAN1 knockout HEK293T cell line (ab266248)

All lanes : Anti-LMAN1 antibody [EPR6979] (**ab125006**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : LMAN1 knockout HEK-293T cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

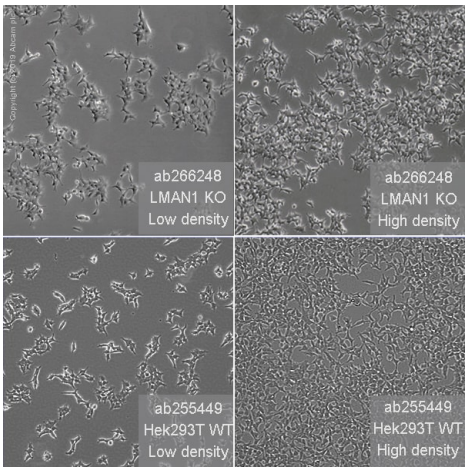
Predicted band size: 58 kDa
Observed band size: 55 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab125006** observed at 55 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab125006 Anti-LMAN1 antibody [EPR6979] was shown to specifically react with Protein ERGIC-53 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266248 (knockout cell lysate **ab257505**) was used. Wild-type and Protein ERGIC-53 knockout samples were subjected to SDS-PAGE. **ab125006** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°CC 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 LMAN1 knockout HEK293T cell line (ab266248)

Homozygous: 1 bp insertion in exon 1



Cell Culture - Human LMAN1 knockout HEK293T cell line (ab266248)

Representative images of LMAN1 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 a EVOS XL Core microscope.

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