abcam

Product datasheet

Human GPI (AMF) knockout HEK-293T cell line ab266834

3 Images

Overview

Product name	Human GPI (AMF) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 76 bp deletion in exon 2
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.
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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
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	Besides it's role as a glycolytic enzyme, mammalian GPI can function as a tumor-secreted cytokine and an angiogenic factor (AMF) that stimulates endothelial cell motility. GPI is also a neurotrophic factor (Neuroleukin) for spinal and sensory neurons.
Pathway	Carbohydrate degradation; glycolysis; D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose: step 2/4.
Involvement in disease	Defects in GPI are the cause of hemolytic anemia non-spherocytic due to glucose phosphate isomerase deficiency (HA-GPID) [MIM:613470]. It is a form of anemia in which there is no abnormal hemoglobin or spherocytosis. It is caused by glucose phosphate isomerase deficiency. Severe GPI deficiency can be associated with hydrops fetalis, immediate neonatal death and neurological impairment.
Sequence similarities	Belongs to the GPI family.
Post-translational modifications	Phosphorylation at Ser-185 by CK2 has been shown to decrease enzymatic activity and may contribute to secretion by a non-classical secretory pathway. lSGylated.
Cellular localization	Cytoplasm. Secreted.

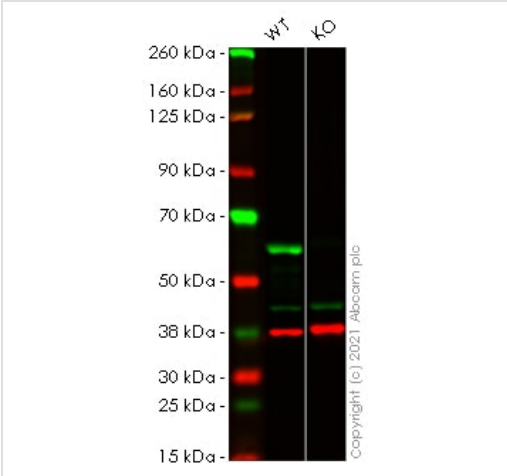
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab266834 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: 63 kDa.

Images



Western blot - Human GPI (AMF) knockout HEK293T cell line (ab266834)

All lanes : Anti-AMF antibody [1B7D7] (ab66340) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : GPI knockout HEK-293T cell lysate

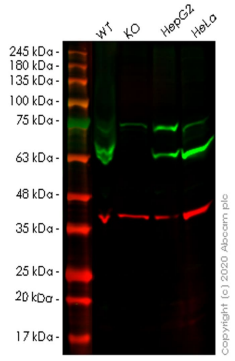
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 63 kDa
Observed band size: 63 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab66340 observed at 63 kDa. Red - loading control ab181602 (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37 kDa.

ab66340 was shown to react with AMF in wild-type HEK-293T cells in Western blot with loss of signal observed in GPI knockout cell line ab266834 (GPI knockout cell lysate ab257458). Wild-type HEK-293T and GPI knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab66340 and ab181602 (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4 °C at a 1 in 1000 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 h at room temperature before imaging.



Western blot - Human GPI knockout HEK293T cell line (ab266834)

All lanes : Anti-AMF antibody [EPR11663(B)] (ab167394) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : GPI knockout HEK293T cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : 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: 63 kDa
Observed band size: 63 kDa

Lanes 1-4: Merged signal (red and green). Green - ab167394 observed at 63 kDa. Red - loading control ab8245 observed at 36 kDa.

ab167394 Anti-AMF antibody [EPR11663(B)] was shown to specifically react with AMF in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266834 (knockout cell lysate ab257458) was used. Wild-type and AMF knockout samples were subjected to SDS-PAGE. ab167394 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2.5 hours 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 GPI knockout HEK293T cell line (ab266834)

Homozygous: 76 bp deletion in exon2

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