abcam

Product datasheet

Human VAMP8 (EDB) knockout A-431 cell line ab269584

2 Images

Overview

Product name	Human VAMP8 (EDB) knockout A-431 cell line
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 4 bp deletion; Frameshift = 99.7%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type A-431 cell line (**ab263975**). 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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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	Skin
Cell type	epithelial
Disease	Epidermoid Carcinoma
Gender	Female
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	Involved in the targeting and/or fusion of transport vesicles to their target membrane. Involved for dense-granule secretion in platelets. Plays a role in regulated enzyme secretion in pancreatic acinar cells. Involved in the abscission of the midbody during cell division, which leads to completely separate daughter cells. Involved in the homotypic fusion of early and late endosomes.
Tissue specificity	Platelets.
Sequence similarities	Belongs to the synaptobrevin family. Contains 1 v-SNARE coiled-coil homology domain.
Cellular localization	Membrane.

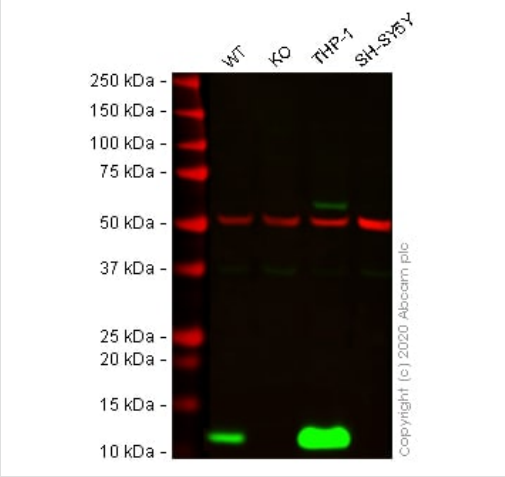
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab269584 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: 11 kDa.

Images



Western blot - Human VAMP8 (EDB) knockout A-431 cell line (ab269584)

All lanes : Anti-VAMP8/EDB antibody [EP2629Y] (**ab76021**) at 1/10000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : VAMP8 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : THP-1 (Human monocytic leukemia cell line) whole cell lysate
Lane 4 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate

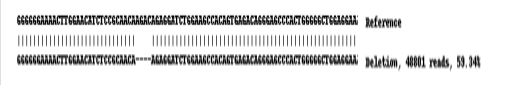
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 11 kDa
Observed band size: 13 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab76021** observed at 13 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab76021 was shown to react with VAMP8/EDB in wild-type A-431 cells in western blot Loss of signal was observed when VAMP8 knockout cell line ab269584 (knockout cell lysate **ab270707**) was used. Wild-type and VAMP8 knockout A-431 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab76021** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Next Generation Sequencing - Human VAMP8 (EDB) knockout A-431 cell line (ab269584)

Knockout achieved by CRISPR/Cas9; X = 4 bp deletion; Frameshift = 99.7%

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