abcam

Product datasheet

Human TGOLN2 (TGN46) knockout A549 cell lysate ab269672

3 Images

Overview

Product name Human TGOLN2 (TGN46) knockout A549 cell lysate

Product overview Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line A549

Organism Human

Mutation description Knockout achieved by CRISPR/Cas9; X = 1 bp deletion, 1 bp insertion; Frameshift: 100%

Passage number <20

Knockout validation Next Generation Sequencing (NGS), Western Blot (WB)

Reconstitution notes

To use as WB control, resuspend the lyophilizate in 50 μL of LDS* Sample Buffer to have a final

concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M

DTT.

*Usage of SDS sample buffer is not recommended with these lyophilized lysates.

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease

inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are

prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -

20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

See here for more information on knockout cell lysates.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of

products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH

Authorisation, and any other relevant authorisations, for their intended uses.

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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**.

Tested applications Suitable for: WB

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Properties

Storage instructions

Store at -80°C. Please refer to protocols.

Components	1 kit
ab280547 - Human TGOLN2 knockout A549 cell lysate	1 x 100μg
ab259782 - Human wild-type A549 cell lysate	1 x 100μg

Cell type epithelial

Disease Carcinoma

Gender Male

Target

Function May be involved in regulating membrane traffic to and from trans-Golgi network.

Tissue specificity Isoform TGN46 is widely expressed. Isoform TGN51 is more abundant in fetal lung and kidney.

Isoform TGN48 is barely expressed in embryonic kidney and promyelocytic cells.

Cellular localization Cell membrane. Golgi apparatus > trans-Golgi network membrane. Primarily in trans-Golgi

network. Cycles between the trans-Golgi network and the cell surface returning via endosomes.

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab269672 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: 51 kDa.

Images

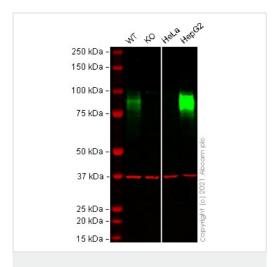
CASTRAGANCOSTUMENDACISTISTOCASTOS COMPACTAMENTACOSTUSTOCIANOCOSTUSTOCIAN DELETINO, 18559 reals, 46.5%

CANTENGANCOTERANGERACUTTCGGATTCCACG-GOTAGCGAGAGGATGACCTTTATCCGAACGGTTCTGGAJ Reference

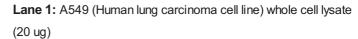
CANTERGRACCOTERAGGRACACTTTCGGATTCCACCGGGTAGCGAGAAGGATGACCTTTATCCGAACGGTTCTGGA Insertion, 14634 reads, 41.04t

Next Generation Sequencing - Human TGOLN2 (TGN46) knockout A549 cell lysate (ab269672)

Knockout achieved by CRISPR/Cas9; X = 1 bp deletion, 1 bp insertion; Frameshift: 100%



Western blot - Human TGOLN2 knockout A549 cell lysate



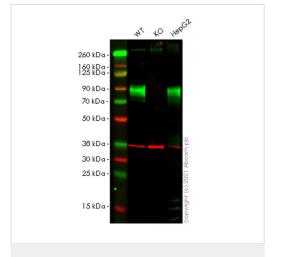
Lane 2: TGOLN2 knockout A549 (Human lung carcinoma cell line) whole cell lysate (20 ug)

Lane 3: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate (20 ug)

Lane 4: Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate (20 ug)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab174280</u> observed at 80 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab174280 was shown to react with TGN46 in wild-type A549 cells in Western blot with loss of signal observed in TGOLN2 knockout cell line ab269510 (knockout cell lysate ab269672). Wild-type A549 and TGOLN2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab174280 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 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 h at room temperature before imaging.



Western blot - Human TGOLN2 knockout A549 cell lysate

Blocking and diluting buffer and concentration: 3% NFDM/TBST. Samples:

Lane 1: Wild-type A549 (human lung carcinoma epithelial cell) whole cell lysate, 40 ug

Lane 2: TGOLN2 (TGN46) knockout A549 (human lung carcinoma epithelial cell) whole cell lysate, 40 ug

Lane 3: HepG2 (human hepatocellar carcinoma epithelial cell) whole cell lysate, 20 ug

Lanes 1-3: Merged signal (red and green).

Green: ab271183 observed at 80-100 kDa.

Red: loading control <u>ab8245</u> (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

Lanes 1-2: <u>ab271183</u> was shown to react with TGN46 in wild-type A549 cells in Western blot with loss of signal observed in TGN46 knockout cell line <u>ab269510</u> (knockout cell lysate ab269672). Wild-type A549 and TGN46 knockout cell lysates were subjected to SDS-PAGE.

<u>ab271183</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated at 4°C overnight at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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