abcam

Product datasheet

Human TLE1 knockout MCF7 cell line ab269498

4 Images

Overview

Product name	Human TLE1 knockout MCF7 cell line
Parental Cell Line	MCF7
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift = 100%
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Next Generation Sequencing (NGS)
Tested applications	**Suitable for:** ICC, WB
Biosafety level	1
General notes	Western blot data suggests that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **Recommended control:** Human wild-type MCF7 cell line (**ab271144**). 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:** MEM + 10% FBS + 0.01 mg/ml bovine insulin **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 5-7x10^4 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 5-7x10^4 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	Breast
Cell type	epithelial
Disease	Adenocarcinoma
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	Transcriptional corepressor that binds to a number of transcription factors. Inhibits NF-kappa-B-regulated gene expression. Inhibits the transcriptional activation mediated by FOXA2, and by CTNNB1 and TCF family members in Wnt signaling. The effects of full-length TLE family members may be modulated by association with dominant-negative AES. Unusual function as coactivator for ESRRG.
Tissue specificity	In all tissues examined, mostly in brain, liver and muscle.
Sequence similarities	Belongs to the WD repeat Groucho/TLE family. Contains 6 WD repeats.
Domain	WD repeat Groucho/TLE family members are characterized by 5 regions, a glutamine-rich Q domain, a glycine/proline-rich GP domain, a central CcN domain, containing a nuclear localization signal, and a serine/proline-rich SP domain. The most highly conserved are the N-terminal Q domain and the C-terminal WD-repeat domain.
Post-translational modifications	Phosphorylated, probably by CDK1. The degree of phosphorylation varies throughout the cell cycle, and is highest at the G2/M transition. Becomes hyperphosphorylated in response to cell differentiation and interaction with HES1 or RUNX1. Ubiquitinated by XIAP/BIRC4.
Cellular localization	Nucleus. Nuclear and chromatin-associated, depending on isoforms and phosphorylation status. Hyperphosphorylation decreases the affinity for nuclear components.

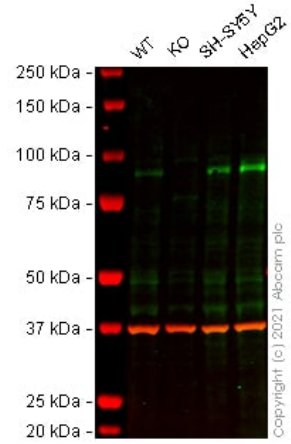
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab269498 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
ICC		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Western blot data suggests that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human TLE1 knockout MCF7 cell line (ab269498)

All lanes : Anti-TLE 1 antibody [EPR9386(2)] (**ab183742**) at 1/1000 dilution

Lane 1 : Wild-type MCF7 (Human breast adenocarcinoma cell line) whole cell lysate
Lane 2 : TLE1 knockout MCF7 (Human breast adenocarcinoma cell line) whole cell lysate
Lane 3 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate
Lane 4 : Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

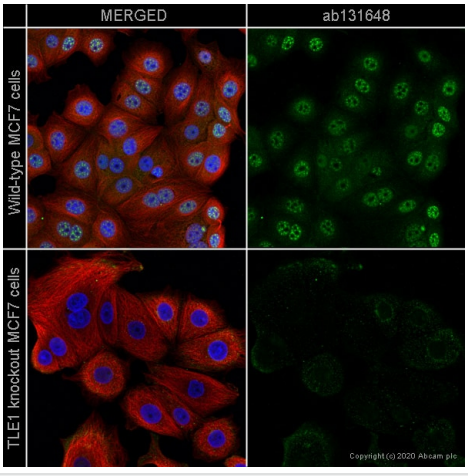
Performed under reducing conditions.

Observed band size: 83 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab183742** observed at 83 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

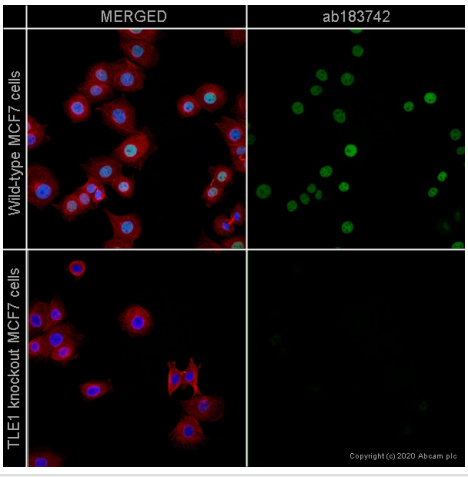
ab183742 was shown to react with TLE 1 in western blot. The band observed in the knockout cell line ab269498 (knockout cell lysate **ab269660**) lane below 83 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab183742** 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.



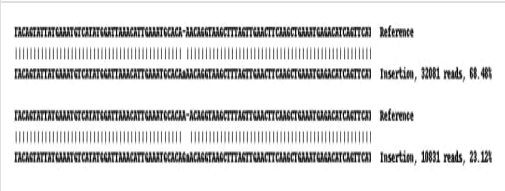
Immunocytochemistry/ Immunofluorescence - Human TLE1 knockout MCF7 cell line (ab269498)

ab131648 staining TLE1 in wild-type MCF7 cells (top panel) and TLE1 knockout MCF7 cells (ab269498) (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab131648** at 1/500 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor®488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor®594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Human TLE1 knockout MCF7 cell line (ab269498)

ab183742 staining TLE1 in wild-type MCF7 cells (top panel) and TLE1 knockout MCF7 cells (ab269498) (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab183742** at 1/500 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a high-content analysis system (Perkin Elmer, Operetta CLS™).



Next Generation Sequencing - Human TLE1 knockout MCF7 cell line (ab269498)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors