

Human TLE1 knockout HEK-293T cell line ab265059

7 图像

概述

产品名称	人TLE1 knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 13 bp deletion in exon 12 and Insertion of the selection cassette in exon 12
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
经测试应用	适用于: ICC/IF, IHC-P, WB
Biosafety level	2
常规说明	<p>Recommended control: Human wild-type HEK293T cell line (ab255593). 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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if</p>

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.

性能

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: 11, 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 15, 20 TH01: 7, 9.3 TPOX: 11, 12 CSF1PO: 12
Mycoplasma free	Yes
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	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.
组织特异性	In all tissues examined, mostly in brain, liver and muscle.
序列相似性	Belongs to the WD repeat Groucho/TLE family. Contains 6 WD repeats.
结构域	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.
翻译后修饰	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.
细胞定位	Nucleus. Nuclear and chromatin-associated, depending on isoforms and phosphorylation status. Hyperphosphorylation decreases the affinity for nuclear components.

应用

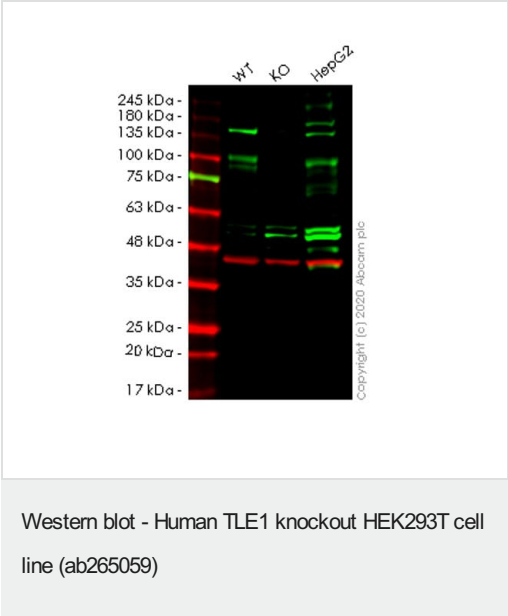
The Abpromise guarantee

Abpromise™承诺保证使用ab265059于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 83 kDa.

图片



All lanes : Anti-TLE 1 antibody [OT11F5] (**ab131648**) at 1/500 dilution

Lane 1 : Wild-type HEK293T cell lysate

Lane 2 : TLE1 knockout HEK293T cell lysate

Lane 3 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

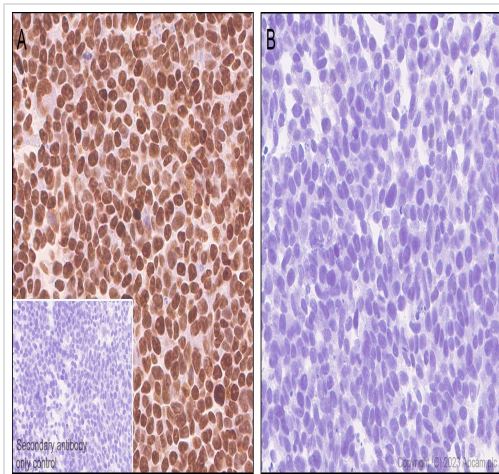
Predicted band size: 83 kDa

Observed band size: 83 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab131648** observed at 83 kDa. Red - loading control, **ab181602** observed at 37 kDa.

ab131648 Anti-TLE 1 antibody [OT11F5] was shown to specifically react with TLE 1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab265059 (knockout cell lysate **ab257240**) was used. Wild-type and TLE 1 knockout samples were subjected to SDS-PAGE. **ab131648** and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary

antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

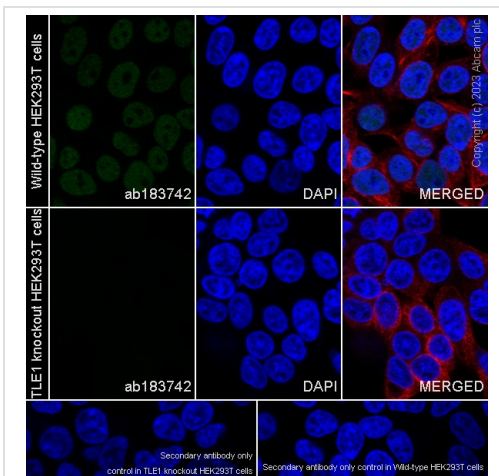


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Human TLE1 knockout HEK-293T cell line (ab265059)

Immunohistochemical analysis of paraffin-embedded fixed (A) Parental HEK293 (Human embryonic kidney epithelial cell) cell pellet (B) TLE1 knockout HEK293 (ab265059) cell pellet, staining TLE 1 with **ab183742** at 1/250 dilution for 30 mins at room temperature. LeicaDS9800 (Bond™ Polymer Refine Detection) used as secondary antibody. Counter-stained using hematoxylin. Positive staining on Wild-type HEK293T cell pellet and no staining on TLE1 knockout HEK293 cell pellet.

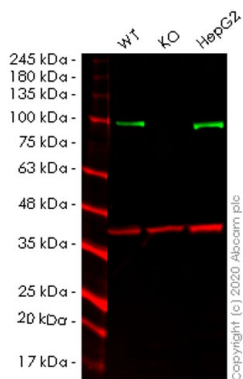
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Human TLE1 knockout HEK-293T cell line (ab265059)

Immunofluorescence analysis of 4% paraformaldehyde-fixed, 0.1% TritonX-100 permeabilised wildtype HEK293T cells and TLE1 knockout HEK293T cells (ab265059) with **ab183742** (green) at 1/50 dilution. Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L (**ab150081**) was used as a secondary antibody, presabsorbed at 1/1000 dilution. Alexa Fluor® 594 Anti-alpha Tubulin mouse monoclonal antibody (**ab195889**) used as microtubule marker counterstain (red). Nuclei were counterstained with DAPI (blue). Confocal image showing nuclear staining in wildtype HEK293T cells and showing no staining in TLE1 knockout HEK293T cells. Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).



Western blot - Human TLE1 knockout HEK293T cell line (ab265059)

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

Lane 1 : Wild-type HEK293T cell lysate

Lane 2 : TLE1 knockout HEK293T cell lysate

Lane 3 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 83 kDa

Observed band size: 83 kDa

Lanes 1-3: Merged signal (red and green). Green - [ab183742](#) observed at 83 kDa. Red - loading control, [ab8245](#) observed at 37 kDa.

[ab183742](#) Anti-TLE 1 antibody [EPR9386(2)] was shown to specifically react with TLE 1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab265059 (knockout cell lysate [ab257240](#)) was used. Wild-type and TLE 1 knockout samples were subjected to SDS-PAGE. [ab183742](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4°C 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 10000 dilution for 1 hour at room temperature before imaging.

Mut	AGCCTCCAGCCATAGAC-----AAGCGGGTACAGCATCGCCTCTTGTGTGC
WT	AGCCTCCAGCCATAGACCCCTCGTTAACC AAGCGGGTACAGCATCGCCTCTTGTGTGC

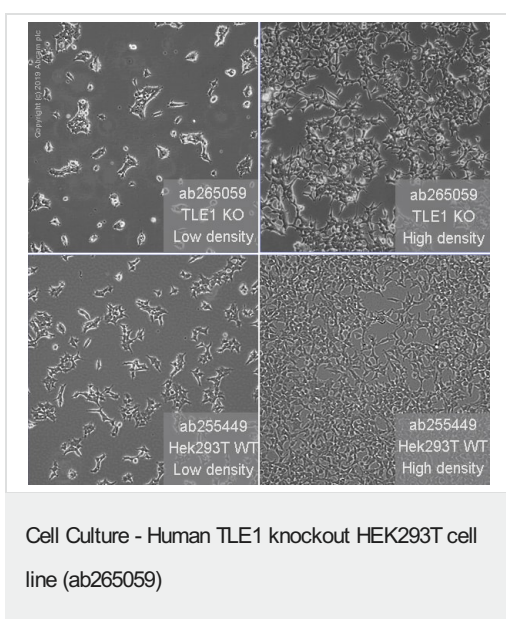
Sanger Sequencing - Human TLE1 knockout
HEK293T cell line (ab265059)

Allele-1: 13 bp deletion in exon 12.

Mut	CATAGACCCCTCGTTAACC*****Insertion*****AAGCGGGTACAGCATCGCCT
WT	CATAGACCCCTCGTTAACC AAGCGGGTACAGCATCGCCT

Sanger Sequencing - Human TLE1 knockout
HEK293T cell line (ab265059)

Allele-2: Insertion of the selection cassette in exon 12.



Representative images of TLE1 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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