abcam

Product datasheet

Anti-TLE 1 antibody [EPR9386(2)] ab183742

KO VALIDATED Recombinant RabMAb

1 Abreviews 7 References 11 Images

Overview

Product name	Anti-TLE 1 antibody [EPR9386(2)]
Description	Rabbit monoclonal [EPR9386(2)] to TLE 1
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: MCF7, HEK-293T, SH-SY5Y, HepG2, Jurkat and HeLa cell lysates. IHC-P: Human schwannoma and synovial sarcoma tissues, HEK-293T cells. ICC/IF: MCF7 and HepG2 cells, HEK-293T cell pellet.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR9386(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab183742 in the following tested applications.

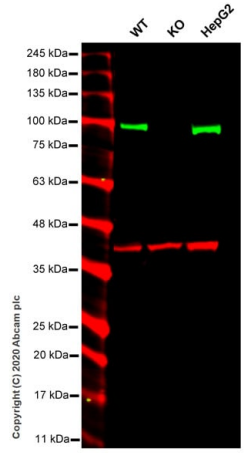
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/100.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		1/1000 - 1/2000. Detects a band of approximately 83 kDa (predicted molecular weight: 83 kDa).

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.

Images



Western blot - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

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

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : TLE1 knockout HEK-293T cell lysate
Lane 3 : HepG2 cell lysate

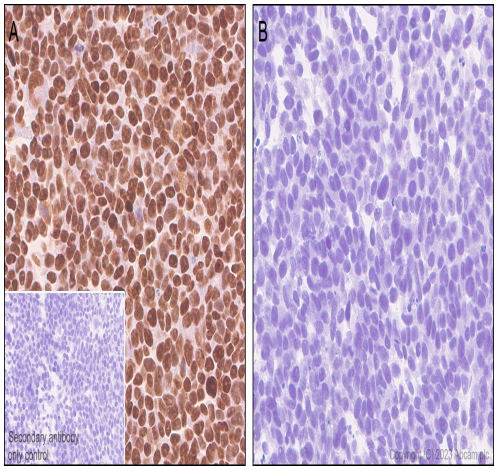
Lysates/proteins at 20 µg per lane.

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.

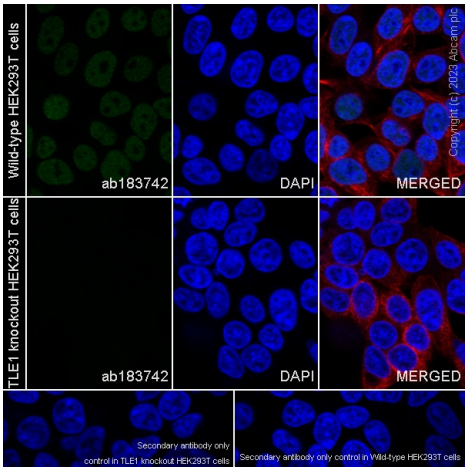
ab183742 Anti-TLE 1 antibody [EPR9386(2)] was shown to specifically react with TLE 1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab265059** (knockout cell lysate **ab257240**) was used. Wild-type and TLE 1 knockout samples were subjected to SDS-PAGE. ab183742 and 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

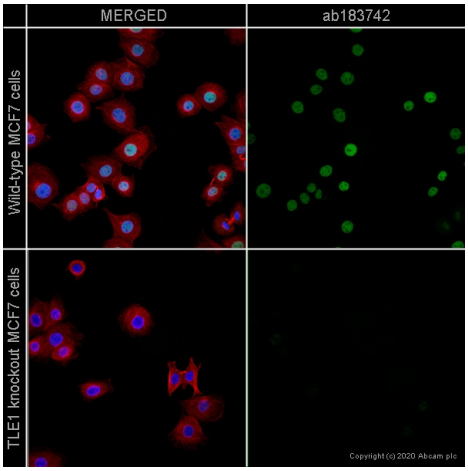
Immunohistochemical analysis of paraffin-embedded fixed (A) Parental HEK293 (Human embryonic kidney epithelial cell) cell pellet (B) TLE1 knockout HEK293 (**ab265059**) cell pellet, staining TLE 1 with ab183742 at 1/250 dilution for 30 mins at room temperature. LeicaDS9800 (Bond™ Polymer Refine Detection) 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.



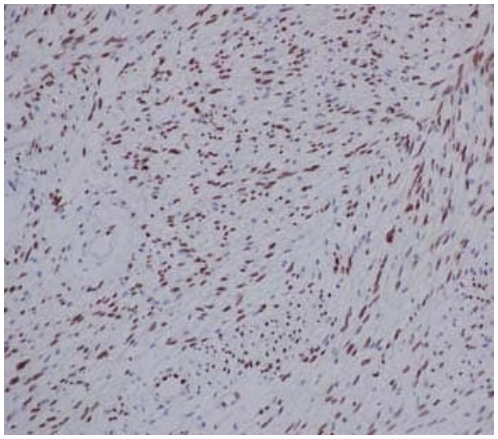
Immunocytochemistry/ Immunofluorescence - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

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).



Immunocytochemistry/ Immunofluorescence - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

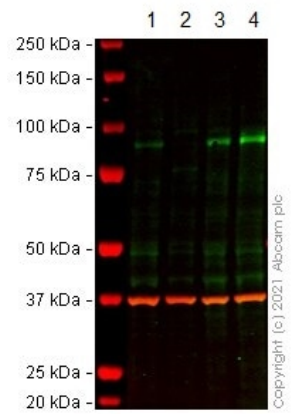
ab183742 staining TLE1 in wild-type MCF7 cells (top panel) and TLE1 knockout MCF7 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. 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™).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

Immunohistochemical analysis of Human schwannoma, staining TLE 1 with ab183742 at 1/250 dilution. Detected using HRP Polymer for Rabbit IgG and counter-stained using hematoxylin.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

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

Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : TLE1 CRISPR/Cas9 edited MCF7 cell lysate
Lane 3 : SH-SY5Y cell lysate
Lane 4 : HepG2 cell lysate

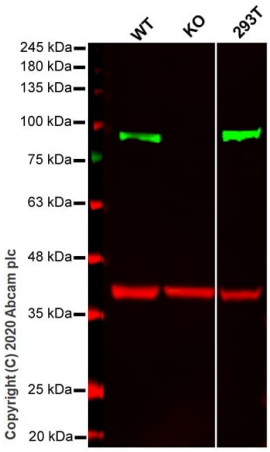
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 83 kDa

Lanes 1 - 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 CRISPR/Cas9 edited lysate 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.



Western blot - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

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

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : TLE1 knockout HeLa cell lysate
Lane 3 : 293T cell lysate

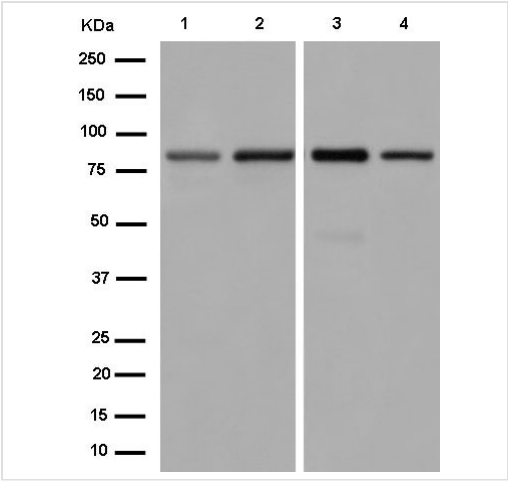
Lysates/proteins at 20 µg per lane.

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 HeLa cells. Loss of signal was observed when knockout cell line **ab264901** (knockout cell lysate **ab257241**) was used. Wild-type and TLE 1 knockout samples were subjected to SDS-PAGE. ab183742 and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 500 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.



Western blot - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

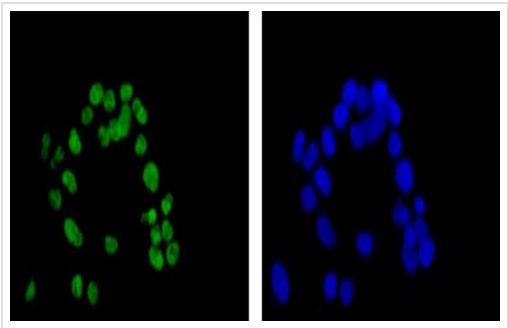
All lanes : Anti-TLE 1 antibody [EPR9386(2)] (ab183742) at 1/1000 dilution

Lane 1 : SH-SY5Y cell lysate
Lane 2 : HepG2 cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

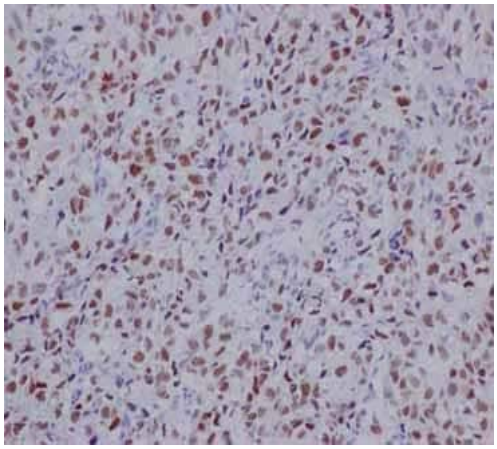
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 83 kDa
Observed band size: 83 kDa



Immunocytochemistry/ Immunofluorescence - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

Immunofluorescence analysis of paraformaldehyde-fixed HepG2 cells, staining TLE 1 (green) with ab183742 at 1/100 dilution. Alexa Fluor®488-conjugated goat anti rabbit IgG was used as a secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

Immunohistochemical analysis of Human synovial sarcoma, staining TLE 1 with ab183742 at 1/250 dilution. Detected using HRP Polymer for Rabbit IgG and counter-stained using hematoxylin.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Anti-TLE 1 antibody [EPR9386(2)] (ab183742)

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