abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

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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. ICC/IF: MCF7 and HepG2 cells.
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.

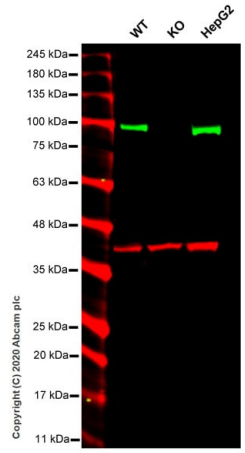
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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

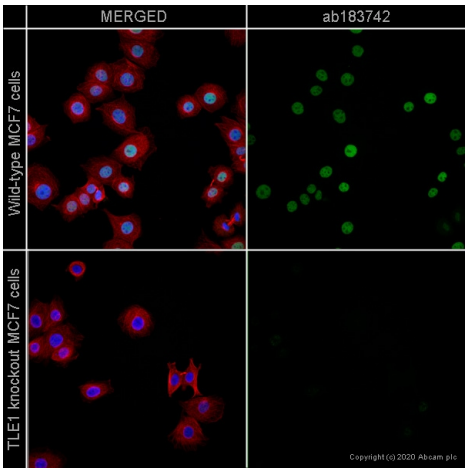
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

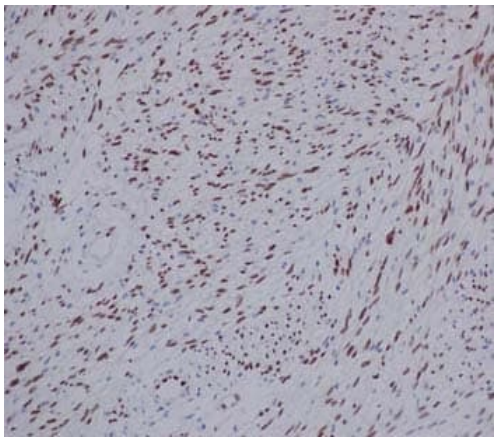
Lanes 1-3: Merged signal (red and green). Green - ab183742 observed at 83 kDa. Red - loading control, **ab8245** observed at 37 kDa.

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.



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

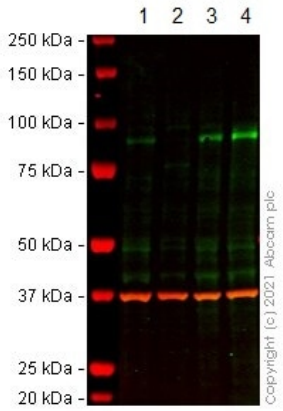
ab183742 staining TLE1 in wild-type MCF7 cells (top panel) and TLE1 knockout MCF7 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h.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

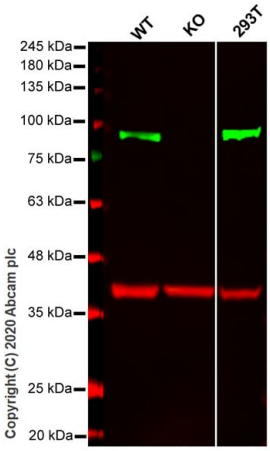
Lysates/proteins at 20 µg per lane.

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

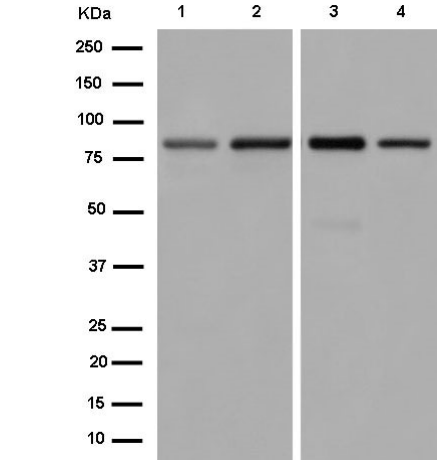
Lysates/proteins at 20 µg per lane.

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)

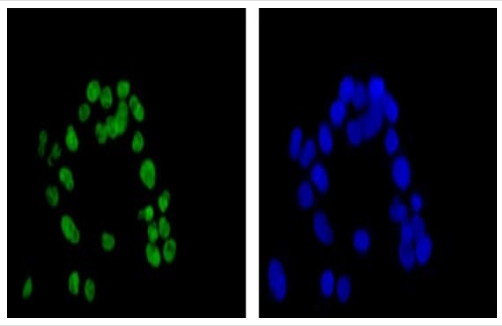
All lanes : Anti-TLE 1 antibody [EPR9386(2)] (ab183742) at 1/1000 dilution

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.

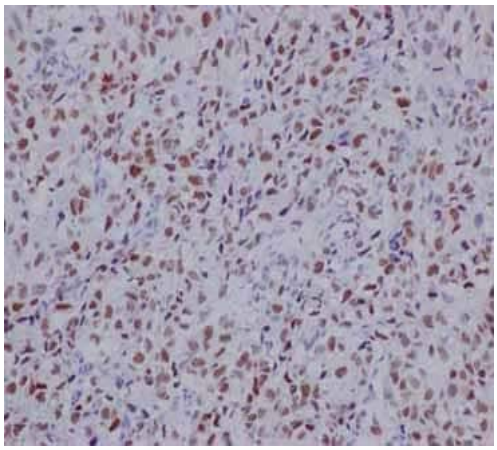
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

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



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

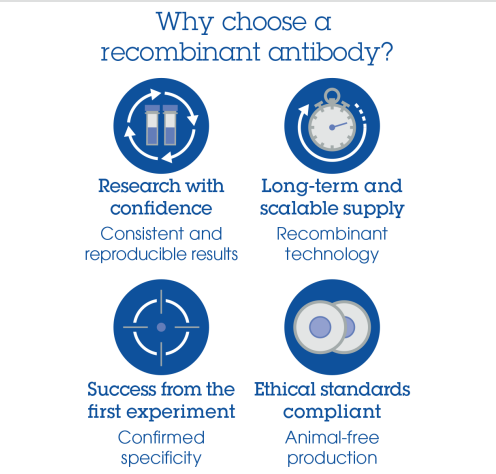
Immunofluorescence analysis of paraformaldehyde-fixed HepG2 cells, staining TLE 1 (green) with ab183742 at 1/100 dilution. Alexa Fluor®488-conjugated goat anti rabbit IgG was used as a secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue).



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)

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