abcam

ab52936

Anti-Tuberin antibody [EP1107Y]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Tissue culture supernatant, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP1107Y
Purification technique	Affinity purification Protein A
Concentration	0.423 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

IP

Tested

Species	Human
Dilution info	1/70
Notes	-

WB

Tested

Species	Human
Dilution info	1/20000 - 1/100000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/40
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information TSC2

Function	Catalytic component of the TSC-TBC complex, a multiprotein complex that acts as a negative regulator of the canonical mTORC1 complex, an evolutionarily conserved

central nutrient sensor that stimulates anabolic reactions and macromolecule biosynthesis to promote cellular biomass generation and growth (PubMed:12172553, PubMed:12271141, PubMed:12842888, PubMed:12906785, PubMed:15340059, PubMed:22819219, PubMed:24529379, PubMed:28215400, PubMed:33436626, PubMed:35772404). Within the TSC-TBC complex, TSC2 acts as a GTPase-activating protein (GAP) for the small GTPase RHEB, a direct activator of the protein kinase activity of mTORC1 (PubMed:12172553, PubMed:12820960, PubMed:12842888, PubMed:12906785, PubMed:15340059, PubMed:22819219, PubMed:24529379, PubMed:33436626). In absence of nutrients, the TSC-TBC complex inhibits mTORC1, thereby preventing phosphorylation of ribosomal protein S6 kinase (RPS6KB1 and RPS6KB2) and EIF4EBP1 (4E-BP1) by the mTORC1 signaling (PubMed:12172553, PubMed:12271141, PubMed:12842888, PubMed:12906785, PubMed:22819219, PubMed:24529379, PubMed:28215400, PubMed:35772404). The TSC-TBC complex is inactivated in response to nutrients, relieving inhibition of mTORC1 (PubMed:12172553, PubMed:24529379). Involved in microtubule-mediated protein transport via its ability to regulate mTORC1 signaling (By similarity). Also stimulates the intrinsic GTPase activity of the Ras-related proteins RAP1A and RAB5 (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Storage information	Stable for 12 months at -20°C

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Species reactivity
Rat: We have preliminary internal testing data to indicate this antibody may not react with this species.
Please contact us for more information.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Tuberin also known as TSC2 is a tumor suppressor protein with a role in cell growth regulation. It has a molecular mass of approximately 200 kDa. This protein forms a critical component of the TSC1-TSC2 complex and is expressed widely in various tissues including the brain kidney heart and skin. Tuberin possesses GTPase-activating protein (GAP) activity targeting specifically the small GTPase protein Rheb which leads to inhibition of the mammalian target of rapamycin complex 1 (mTORC1).
Biological function summary	Tuberin helps control cell size and proliferation. It does this by modulating the mTOR signaling pathway. This modulation is essential for maintaining cellular homeostasis and energy balance particularly under conditions of nutrient scarcity. Tuberin as part of the TSC1-TSC2 complex also influences cell cycle progression and protein synthesis. Its functions are vital in preventing abnormal cell growth and division which is particularly important in organs where rapid cell turnover occurs.
Pathways	Tuberin plays a central role in the mTOR signaling pathway which affects cell growth autophagy and metabolism. It directly interacts with hamartin (TSC1) to form the TSC1-TSC2 complex which regulates Rheb activity. By inhibiting Rheb Tuberin helps control the mTORC1 pathway linking it to signals such as growth factors stress energy status and amino acid availability. This coordination is important for the proper balance between cell growth and catabolism.
Associated diseases and disorders	Tuberin is mainly associated with Tuberous Sclerosis Complex (TSC) and certain forms of epilepsy. Mutations or loss of function in TSC2 can lead to benign tumor formations in multiple organs including the brain kidneys and skin. It is also implicated in the development of lymphangioleiomyomatosis (LAM) a rare lung disease. Tuberin's interaction with mTORC1 highlights its connection with these conditions as hyperactivation of the mTOR pathway contributes significantly to the pathogenesis.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

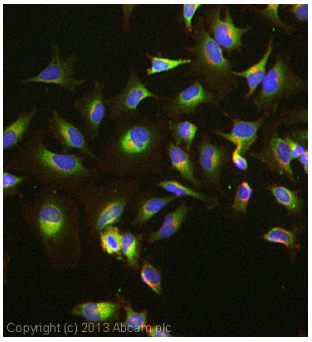
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

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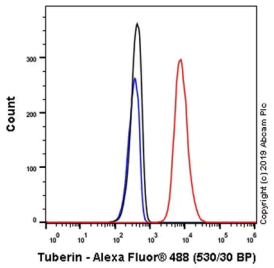
10 product images



Immunocytochemistry/ Immunofluorescence - Anti-Tuberin antibody [EP1107Y] (ab52936)

Tuberin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Tuberin antibody

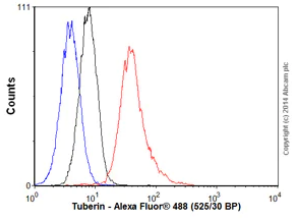
ab52936 stained HeLa cells. The cells were 4% formaldehyde fixed for 10 minutes at room temperature and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab52936 at 1/100 dilution) overnight at +4°C. The secondary antibody (pseudo-colored green) was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) used at a 1/1000 dilution for 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Flow Cytometry (Intracellular) - Anti-Tuberin antibody [EP1107Y] (ab52936)

Tuberin Flow Cytometry (Intracellular) staining using rabbit Anti-Tuberin antibody

Overlay histogram showing SH-SY5Y (Human neuroblastoma epithelial cell) cells stained with ab52936 (red line). The cells were fixed with 4% Paraformaldehydeand then permeabilized with 90% Methanol. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit IgG (H&L) ab150077 at 1/2000 dilution. Isotype control antibody (black line) was rabbit IgG (monoclonal). Unlabelled sample (blue line) was Cell without incubation with primary antibody and secondary antibody.

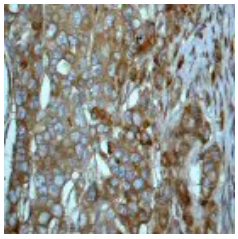


Flow Cytometry (Intracellular) - Anti-Tuberin antibody [EP1107Y] (ab52936)

Tuberin Flow Cytometry (Intracellular) staining using rabbit Anti-Tuberin antibody

Overlay histogram showing HeLa cells stained with ab52936 (red line). The cells were fixed with 80% methanol (5 min)/ and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab52936 1/10000 dilution) for 30 min at 22°C. The

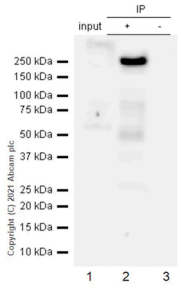
secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 4% formaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tuberin antibody [EP1107Y] (ab52936)

Tuberin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Tuberin antibody

Immunohistochemical analysis of paraffin-embedded human adenocarcinoma of uterus using ab52936 at a dilution of 1/100-1/250.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-Tuberin antibody [EP1107Y] (ab52936)

Tuberin was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with ab52936 at 1/50 dilution (2µg). VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.
Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg

Lane 2: ab52936 IP in HeLa whole cell lysate

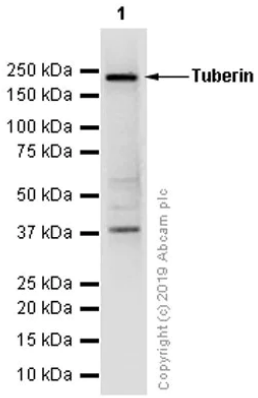
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab52936 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-Tuberin antibody [EP1107Y] (ab52936)

Predicted band size: 201 kDa

Observed band size: 200 kDa



Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936)

Blocking/Diluting Buffer and concentration:
5% NFDM/TBST
All lanes:
Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936) at 1/20000 dilution

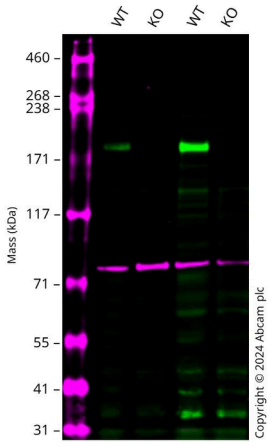
All lanes:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate prepared in 1%SDS Hot lysis method at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 201 kDa

Observed band size: 200 kDa



Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936)

Tuberin Western blot staining using rabbit Anti-Tuberin antibody

Western blot: Anti-TSC2 antibody [EP1107Y] (ab52936) staining at 1/50000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab52936 was shown to bind specifically to TSC2. A band was observed at 200 kDa in wild-type A549 cell lysates with no signal observed at this size in TSC2 knockout cell line. To generate this image, wild-type and TSC2 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936) at 1/50000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
TSC2 knockout A549 cell lysate at 20 µg

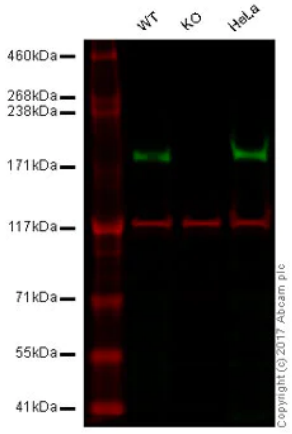
Lane 3:
Wild-type HAP1 cell lysate at 20 µg

Lane 4:
TSC2 knockout HAP1 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.



Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936)

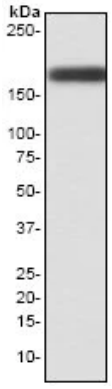
Tuberin Western blot staining using rabbit Anti-Tuberin antibody

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: Tuberin knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab52936 observed at 180 kDa. Red - loading control, ab18058, observed at 130 kDa.
ab52936 was shown to specifically react with tuberin in wild-type HAP1 cells. No band was observed when tuberin knockout samples were used. Wild-type and tuberin knockout samples were subjected to SDS-PAGE. ab52936 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed 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 1/10000 dilution for 1 hour at room temperature before imaging.
All lanes:
Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936)

Predicted band size: 201 kDa



Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936)

The cell lysates was prepared in 1%SDS Hot lysis method.
All lanes:
Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936) at 1/100000 dilution

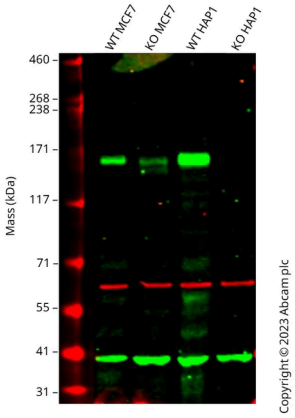
All lanes:
SH-SY5Y cell lysate at 10 µg

Secondary

All lanes:
HRP-labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 201 kDa

Observed band size: ~200 kDa



Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936)

Tuberin Western blot staining using rabbit Anti-Tuberin antibody

Anti-TSC2 antibody [EP1107Y] (ab52936) staining at 1/20000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab52936 detected a band observed at 200 kDa in wild-type MCF7 cell lysates with no change observed in the TSC2 knockout cell line ab286525. To generate this image, wild-type and TSC2 knockout MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-Tuberin antibody [EP1107Y] (ab52936) at 1/20000 dilution

Lane 1:
Wild-type MCF7 cell lysate at 20 µg

Lane 2:
Western blot - Human TSC2 knockout MCF7 cell line (ab286525) at 20 µg

Lane 2:
TSC2 knockout MCF7 cell lysate at 20 µg

Lane 3:
Wild-type HAP1 cell lysate at 20 µg

Lane 4:
TSC2 knockout HAP1 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 201 kDa

Observed band size: 200 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.