

Product datasheet

Anti-Tuberin antibody [EP1107Y] α b52936

KO VALIDATED

Recombinant

RabMAb

[4 References](#) [9 Images](#)

Overview

Product name	Anti-Tuberin antibody [EP1107Y]
Description	Rabbit monoclonal [EP1107Y] to Tuberin
Host species	Rabbit
Tested applications	Suitable for: WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide within Human Tuberin aa 1750-1850. The exact sequence is proprietary.
Positive control	IHC-P: Human adenocarcinoma of uterus. IF/ICC: HeLa cell line. WB: SH-SY5Y and HeLa cell lysate. Flow Cyt (intra): SH-SY5Y and HeLa cells. IP: HeLa cells.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant</p>
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EP1107Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52936 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
WB		1/20000 - 1/100000. Detects a band of approximately 201 kDa (predicted molecular weight: 201 kDa).
IP		1/70.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100.
Flow Cyt (Intra)		1/40. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function	In complex with TSC1, inhibits the nutrient-mediated or growth factor-stimulated phosphorylation of S6K1 and EIF4EBP1 by negatively regulating mTORC1 signaling. Acts as a GTPase-activating protein (GAP) for the small GTPase RHEB, a direct activator of the protein kinase activity of mTORC1. Implicated as a tumor suppressor. Involved in microtubule-mediated protein transport, but this seems to be due to unregulated mTOR signaling. Stimulates weakly the intrinsic GTPase activity of the Ras-related proteins RAP1A and RAB5 in vitro. Mutations in TSC2 lead to constitutive activation of RAP1A in tumors.
Tissue specificity	Liver, brain, heart, lymphocytes, fibroblasts, biliary epithelium, pancreas, skeletal muscle, kidney, lung and placenta.
Involvement in disease	<p>Defects in TSC2 are the cause of tuberous sclerosis type 2 (TSC2) [MIM:613254]. TSC2 is an autosomal dominant multi-system disorder that affects especially the brain, kidneys, heart, and skin. It is characterized by hamartomas (benign overgrowths predominantly of a cell or tissue type that occurs normally in the organ) and hamartias (developmental abnormalities of tissue combination). Clinical symptoms can range from benign hypopigmented macules of the skin to profound mental retardation with intractable seizures to premature death from a variety of disease-associated causes.</p> <p>Defects in TSC2 are a cause of lymphangioleiomyomatosis (LAM) [MIM:606690]. LAM is a progressive and often fatal lung disease characterized by a diffuse proliferation of abnormal smooth muscle cells in the lungs. It affects almost exclusively young women and can occur as an isolated disorder or in association with tuberous sclerosis complex.</p>
Sequence similarities	Contains 1 Rap-GAP domain.

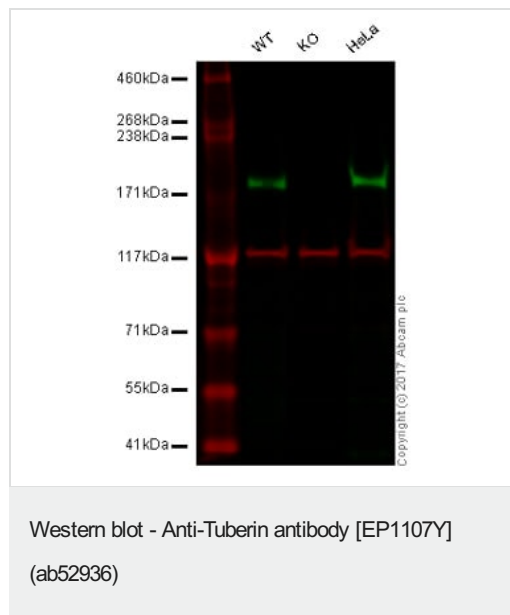
**Post-translational
modifications**

Cellular localization

Phosphorylation at Ser-1387, Ser-1418 or Ser-1420 does not affect interaction with TSC1.
Phosphorylation at Ser-939 and Thr-1462 by PKB/AKT1 is induced by growth factor stimulation.

Cytoplasm. Membrane. At steady state found in association with membranes.

Images



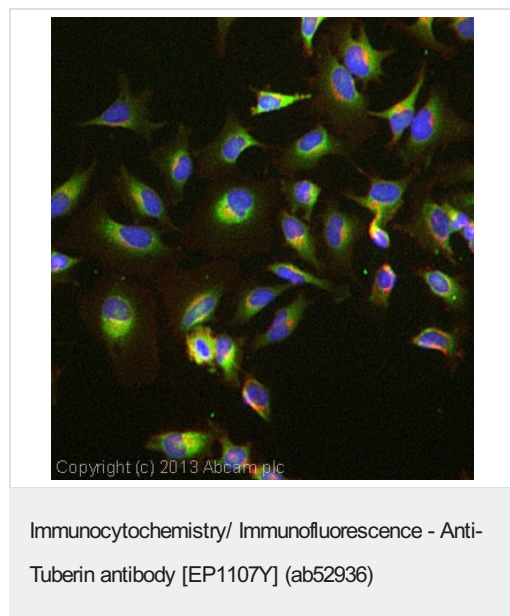
Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: Tuberin knockout HAP1 whole cell lysate (20 µg)

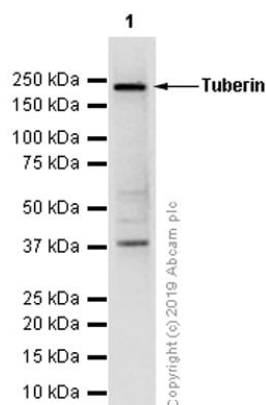
Lane 3: HeLa whole cell lysate (20 µg)

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.



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.



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

Anti-Tuberin antibody [EP1107Y] (ab52936) at 1/20000 dilution +
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell
lysate prepared in 1%SDS Hot lysis method at 15 µg

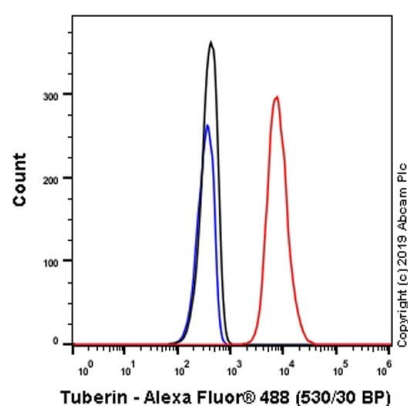
Secondary

Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 201 kDa

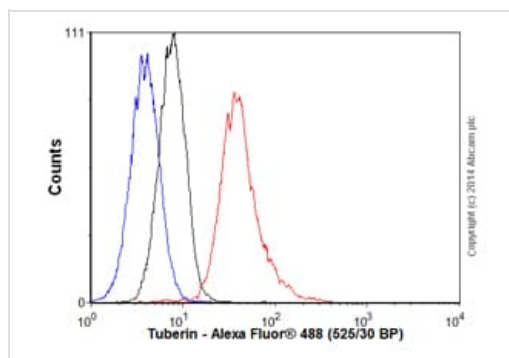
Observed band size: 200 kDa

Blocking/Diluting Buffer and concentration:
5% NFDM/TBST



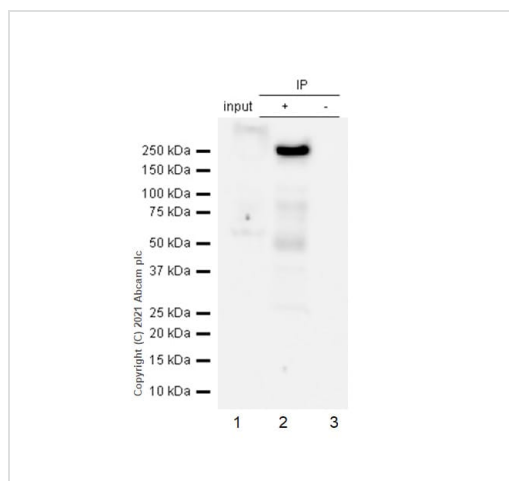
Flow Cytometry (Intracellular) - Anti-Tuberin antibody
[EP1107Y] (ab52936)

Overlay histogram showing SH-SY5Y (Human neuroblastoma
epithelial cell) cells stained with ab52936 (red line). The cells were
fixed with 4% Paraformaldehyde and then permeabilized with 90%
Methanol. The secondary antibody used was Alexa Fluor® 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)

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/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 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.



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

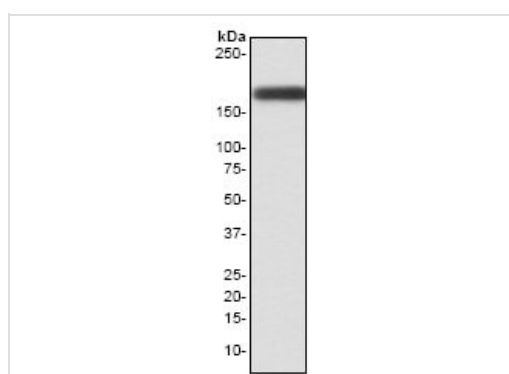
Tuberin was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with ab52936 at 1/50 dilution (2µg). VeriBlot for IP Detection Reagent (HRP)([ab131366](#)) was used at 1/5000 dilution.

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.



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

Anti-Tuberin antibody [EP1107Y] (ab52936) at 1/100000 dilution + SH-SY5Y cell lysate at 10 µg

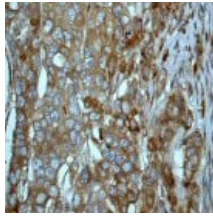
Secondary

HRP-labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 201 kDa

Observed band size: ~200 kDa

The cell lysates was prepared in 1%SDS Hot lysis method.

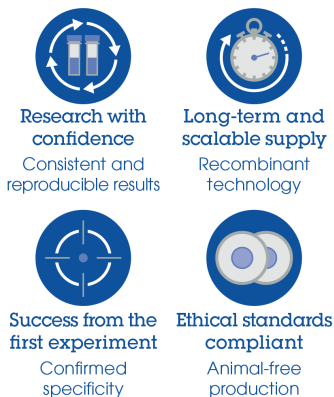


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tuberin antibody [EP1107Y] (ab52936)

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.

Why choose a recombinant antibody?



Anti-Tuberin antibody [EP1107Y] (ab52936)

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