abcam

# ab40872

# Anti-Hamartin antibody [EP318Y]

Recombinant | RabMAb | KO Validated

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 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** | EP318Y |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.216 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

### WB

#### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 |
| **Notes** | For the unpurified version use 1/20000 dilution |

### IHC-P

**Tested**

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/200 |
| **Notes** | - |

## ICC/IF

**Not recommended**

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | For unpurified version use 1/500 dilution |

## Flow Cyt (Intra)

**Tested**

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/20 |
| **Notes** | ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For the unpurified version use 1/1000- 1/10000 dilution |

## IP

**Tested**

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/500 |
| **Notes** | - |

# Target data

See full target information TSC1

**Function** Non-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:12906785, PubMed:15340059, PubMed:24529379, PubMed:28215400). The TSC-TBC complex acts as a GTPase-activating protein (GAP) for the small GTPase RHEB, a direct activator of the protein kinase activity of mTORC1 (PubMed:12906785, PubMed:15340059, PubMed:24529379). 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:12271141, PubMed:24529379, PubMed:28215400, PubMed:33215753). The TSC-TBC complex is inactivated in response to nutrients, relieving inhibition of mTORC1 (PubMed:12172553, PubMed:24529379). Within the TSC-TBC complex, TSC1 stabilizes TSC2 and prevents TSC2 self-aggregation (PubMed:10585443, PubMed:28215400). Acts as a tumor suppressor (PubMed:9242607). Involved in microtubule-mediated protein transport via its ability to regulate mTORC1 signaling (By similarity). Also acts as a co-chaperone for HSP90AA1 facilitating HSP90AA1 chaperoning of protein clients such as kinases, TSC2 and glucocorticoid receptor NR3C1 (PubMed:29127155). Increases ATP binding to HSP90AA1 and inhibits HSP90AA1 ATPase activity (PubMed:29127155). Competes with the activating co-chaperone AHSA1 for binding to HSP90AA1, thereby providing a reciprocal regulatory mechanism for chaperoning of client proteins (PubMed:29127155). Recruits TSC2 to HSP90AA1 and stabilizes TSC2 by preventing the interaction between TSC2 and ubiquitin ligase HERC1 (PubMed:16464865, PubMed:29127155).

## Storage

| | |
|---|---|
| **Shipped at conditions** | Conditional Ambient |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## 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.

## Supplementary info

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

| | |
|---|---|
| **Activity summary** | The protein 'Hamartin' also known as TSC1 plays a mechanical role in cellular processes primarily as a regulator of cellular growth and proliferation. It interacts with Tuberin (TSC2) to form a complex that inhibits the small GTPase Rheb thereby controlling the mTOR signaling pathway. Hamartin has a molecular mass of approximately 130 kDa. It is expressed in various tissues including the brain heart and kidneys highlighting its significance in multiple physiological processes. |
| **Biological function summary** | Hamartin functions as part of the TSC1-TSC2 complex which acts as a tumor suppressor. This complex inhibits the mechanistic target of rapamycin complex 1 (mTORC1) an essential regulator of cell growth proliferation and protein synthesis. Through this regulation Hamartin plays a critical role in maintaining cellular homeostasis and preventing abnormal cell growth that can lead to tumorigenesis. |
| **Pathways** | Hamartin is integral to the mTOR signaling pathway an important pathway that regulates cell metabolism growth and survival. This pathway involves the interaction of Hamartin with Tuberin (TSC2) helping to control the activation of mTORC1. Additionally Hamartin is involved in the PI3K/Akt signaling pathway which further influences mTOR activity and cellular responses to growth factors. |
| **Associated diseases and disorders** | Disruptions in Hamartin function are linked to Tuberous Sclerosis Complex (TSC) a genetic disorder characterized by tumor development in multiple organs. Mutations in the TSC1 gene lead to the loss of Hamartin function resulting in uncontrolled cell growth. Additionally research indicates that Hamartin may have a connection to certain epilepsy types given its expression in the brain and its role in neuronal development. In these conditions the interaction of Hamartin with Tuberin is essential for regulating cellular behavior and preventing disease progression. |

## 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.

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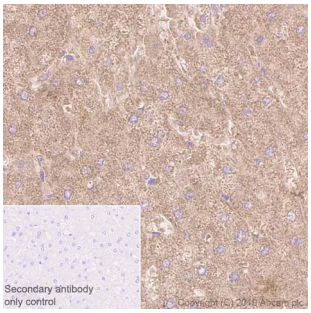
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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

Full details and terms and conditions can be found here:
Terms & Conditions.

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## 7 product images

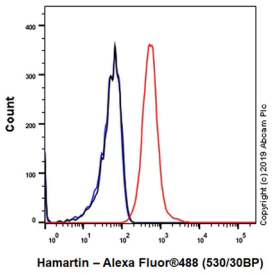



### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hamartin antibody [EP318Y] (ab40872)

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human liver tissue sections labeling Hamartin with purified ab40872 at 1/200 dilution (1.08 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

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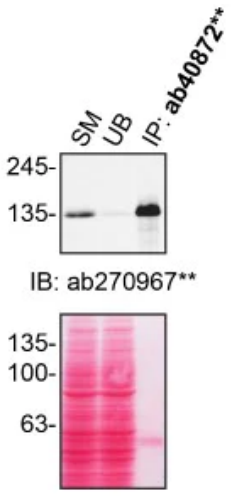



### Flow Cytometry (Intracellular) - Anti-Hamartin antibody [EP318Y] (ab40872)

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

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Hamartin with Purified ab40872 at 1/20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488 ,ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

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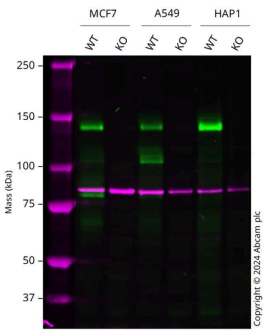



# Immunoprecipitation - Anti-Hamartin antibody [EP318Y] (ab40872)

Immunoprecipitation of TSC1 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 2.0 µg of ab40872 pre-coupled to Protein A beads. Samples were then washed and processed for western blot.

These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Immunoprecipitation - Anti-Hamartin antibody [EP318Y] (ab40872) at 2 µg

All lanes:
HAP1 cells




## Western blot - Anti-Hamartin antibody [EP318Y] (ab40872)

Hamartin Western blot staining using rabbit Anti-Hamartin antibody

Western blot: Anti-TSC1 antibody [EP318Y] (ab40872) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab40872 was shown to bind specifically to TSC1. A band was observed at 130-150 kDa in wild-type MCF7 cell lysates with no signal observed at this size in TSC1 knockout cell line. To generate this image, wild-type and TSC1 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 5 % 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-Hamartin antibody [EP318Y] (ab40872) at 1/1000 dilution

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

Lane 2:
TSC1 knockout MCF7 cell lysate at 20 µg

Lane 3:
Wild-type A549 cell lysate at 20 µg

Lane 4:
TSC1 knockout A549 cell lysate at 20 µg

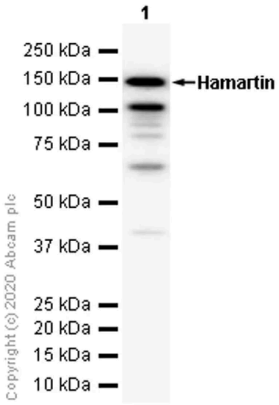
Lane 5:
Wild-type HAP1 cell lysate at 20 µg

Lane 6:
TSC1 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-Hamartin antibody [EP318Y] (ab40872)

Hamartin Western blot staining of HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate using rabbit Anti-Hamartin antibody

We are not sure about the nature of the extra band.
All lanes:
Western blot - Anti-Hamartin antibody [EP318Y] (ab40872) at 1/1000 dilution

All lanes:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 µg

Secondary

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

Predicted band size: 130 kDa



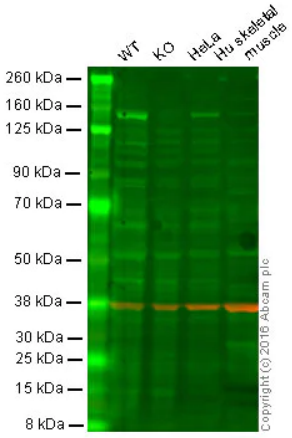

## Western blot - Anti-Hamartin antibody [EP318Y] (ab40872)

ab40872 was shown to react with TSC1 in wild-type HAP1 cells in Western blot with loss of signal observed in a TSC1 knockout cell line. Wild-type HAP1 and TSC1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab40872 overnight at 4 °C at a 1/500 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Western blot - Anti-Hamartin antibody [EP318Y] (ab40872) at 1/500 dilution

Lane 1:
Wild-type HAP1 lysate at 100 µg

Lane 2:
TSC1 knock-out HAP1 lysate at 100 µg




## Western blot - Anti-Hamartin antibody [EP318Y] (ab40872)

Hamartin Western blot staining using rabbit Anti-Hamartin antibody

**Lane 1:** Wild-type HAP1 cell lysate (20 µg)
**Lane 2:** Hamartin knockout HAP1 cell lysate (20 µg)
**Lane 3:** HeLa cell lysate (20 µg)
**Lane 4:** Human skeletal muscle tissue lysate (20 µg)
**Lanes 1 - 4:** Merged signal (red and green). Green - ab40872 observed at 150 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab40872 was shown to recognize Hamartin when Hamartin knockout samples were used, along with additional cross-reactive bands. Wild-type and Hamartin knockout samples were subjected to SDS-PAGE. ab40872 and ab8245 (loading control to GAPDH) were diluted 1/5000 and 1/10000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Hamartin antibody [EP318Y] (ab40872)

Predicted band size: 130 kDa

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