# abcam

### Product datasheet

# Anti-Hamartin antibody [EP318Y] ab40872





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#### Overview

**Product name** Anti-Hamartin antibody [EP318Y]

**Description** Rabbit monoclonal [EP318Y] to Hamartin

**Host species** Rabbit

**Tested applications** Suitable for: WB, IHC-P, Flow Cyt (Intra)

Unsuitable for: ICC/IF

Species reactivity Reacts with: Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: human liver tissue WB: HeLa cells; wild type HAP1 cell lysate, Hamartin knockout HAP1

cell lysate, HeLa cells, Human skeletal muscle tissue lysate Flow Cyt (intra): HeLa 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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS

**Purity** Protein A purified

Clonality Monoclonal Clone number **EP318Y** 

Isotype lgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab40872 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		1/1000. Predicted molecular weight: 150 kDa. For the unpurified version use 1/20000 dilution
IHC-P		1/200. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/20.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody. For the unpurified version use 1/1000- 1/10000 dilution

#### **Application notes**

Is unsuitable for ICC/IF.

#### **Target**

**Function** 

In complex with TSC2, inhibits the nutrient-mediated or growth factor-stimulated phosphorylation of S6K1 and EIF4EBP1 by negatively regulating mTORC1 signaling. Seems not to be required for TSC2 GAP activity towards RHEB. Implicated as a tumor suppressor. Involved in microtubule-mediated protein transport, but this seems to be due to unregulated mTOR signaling.

Tissue specificity

Highly expressed in skeletal muscle, followed by heart, brain, placenta, pancreas, lung, liver and kidney. Also expressed in embryonic kidney cells.

Involvement in disease

Defects in TSC1 are the cause of tuberous sclerosis type 1 (TSC1) [MIM:191100]. It is an autosomal dominant multi-system disorder that affects especially the brain, kidneys, heart, and skin. TS1C 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.

Defects in TSC1 may be a cause of focal cortical dysplasia of Taylor balloon cell type (FCDBC) [MIM:607341]. FCDBC is a subtype of cortical displasias linked to chronic intractable epilepsy. Cortical dysplasias display a broad spectrum of structural changes, which appear to result from changes in proliferation, migration, differentiation, and apoptosis of neuronal precursors and neurons during cortical development.

Domain

The C-terminal putative coiled-coil domain is necessary for interaction with TSC2.

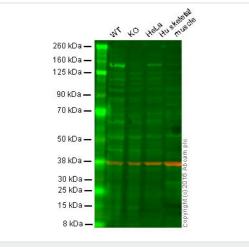
Post-translational modifications

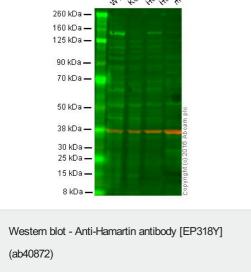
 $Phosphorylation\ at\ Ser\mbox{-}505\ does\ not\ affect\ interaction\ with\ TSC2.\ Phosphorylated\ upon\ DNA$ 

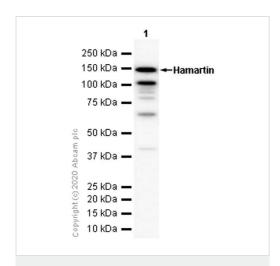
damage, probably by ATM or ATR.

**Cellular localization** Cytoplasm. Membrane. At steady state found in association with membranes.

#### **Images**







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

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 lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

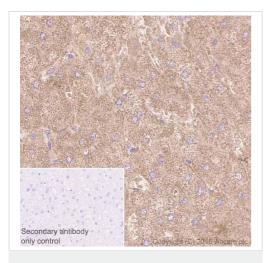
Anti-Hamartin antibody [EP318Y] (ab40872) at 1/1000 dilution (Purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 µg

#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG (H+L), Peroxidase conjugated)

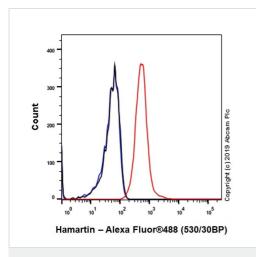
Predicted band size: 150 kDa

We are not sure about the nature of the extra band.



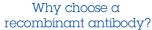
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hamartin antibody
[EP318Y] (ab40872)

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.



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

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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Success from the first experiment

Confirmed specificity

Ethical standards compliant Animal-free production

Anti-Hamartin antibody [EP318Y] (ab40872)

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