abcam

Product datasheet

Anti-Hamartin antibody [EP318Y] ab40872

KO VALIDATED Recombinant RabMAb

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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, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide corresponding to C-terminal residues of human Hamartin.
Positive control	ICC/IF: 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: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	EP318Y
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab40872** in the following tested applications.

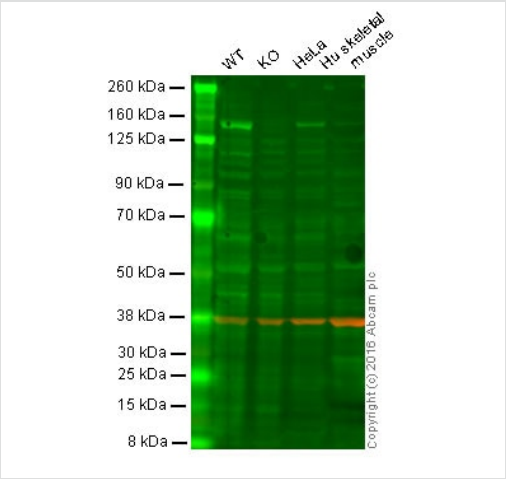
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/20000. Predicted molecular weight: 150 kDa.
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		1/500.
Flow Cyt	★★★☆☆	1/1000 - 1/10000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

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-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.

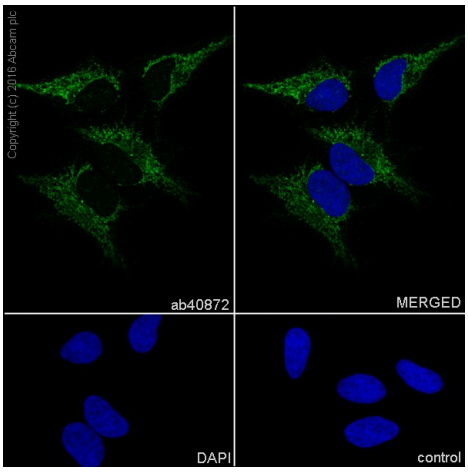
Images



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.

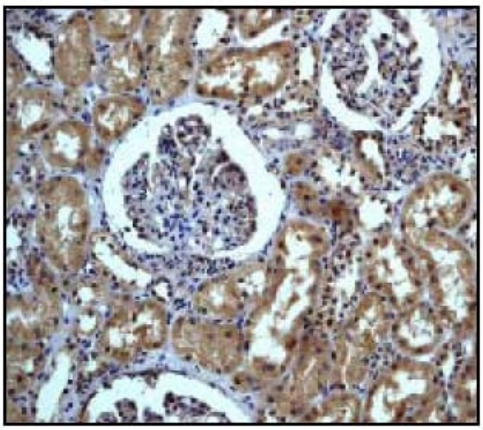
ab40872 was shown to recognize Hamartin when Hamartin knockout samples were used, along with additional cross-reactive bands. Wild-type and Hamartin knockout samples were subjected to SDS-PAGE. ab40872 and ab8245 (loading control to GAPDH) were diluted 1/5000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit 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.



Immunocytochemistry/ Immunofluorescence - Anti-Hamartin antibody [EP318Y] (ab40872)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (human cervix adenocarcinoma epithelial cell) labeling Hamartin with ab40872 at 1/500. ab150077, AlexaFluor®488 Goat anti-Rabbit at 1/1000 was used as the secondary antibody. Cells were fixed with 100% Methanol. DAPI (blue) was used a nuclear counter stain.

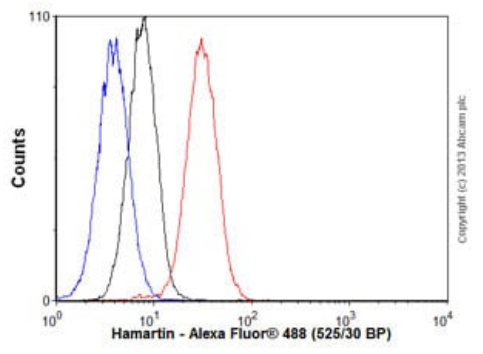
Confocal image showing cytoplasmic staining on HeLa cell line.



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

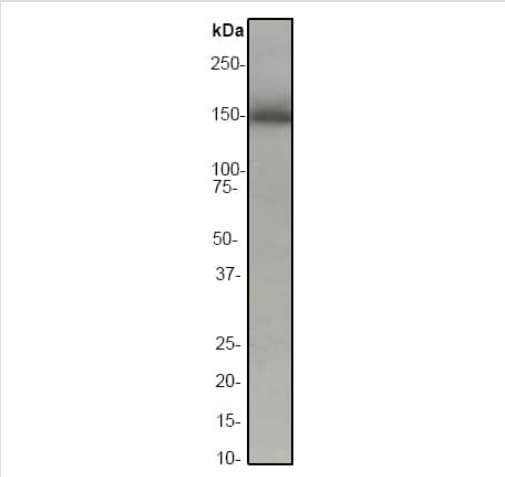
Ab40872 staining human Hamartin in human kidneys by immunohistochemistry using paraffin embedded tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

Overlay histogram showing HeLa cells stained with ab40872 (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 (ab40872, 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 >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% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



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

Anti-Hamartin antibody [EP318Y] (ab40872) at 1/20000 dilution + HeLa cell lysate

Predicted band size: 150 kDa

Observed band size: 150 kDa

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