abcam

Product datasheet

Anti-Hamartin antibody [EP318Y] - BSA and Azide free ab247297

KO VALIDATED Recombinant RabMAb

6 Images

Overview

Product name	Anti-Hamartin antibody [EP318Y] - BSA and Azide free
Description	Rabbit monoclonal [EP318Y] to Hamartin - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, 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	ab247297 is the carrier-free version of **ab40872**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP318Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab247297 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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 150 kDa.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF.

Target

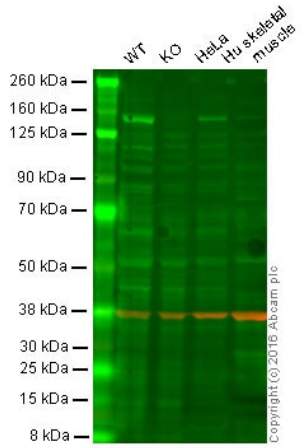
Function In complex with TSC2, inhibits the nutrient-mediated or growth factor-stimulated phosphorylation of S6K1 and EIF4EBP1 by negatively regulating mTORC1 signaling. Seems not to be required for TSC2 GAP activity towards RHEB. Implicated as a tumor suppressor. Involved in microtubule-mediated protein 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-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] - BSA and Azide free (ab247297)

This data was developed using **ab40872**, the same antibody clone in a different buffer formulation.

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

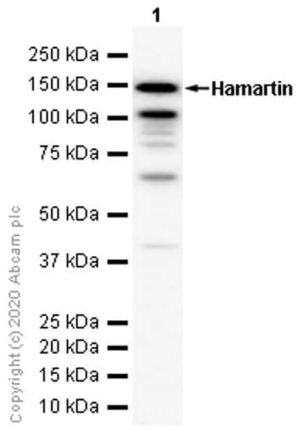
Lane 2: Hamartin knockout HAP1 cell lysate (20 µg)

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.



Western blot - Anti-Hamartin antibody [EP318Y] - BSA and Azide free (ab247297)

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

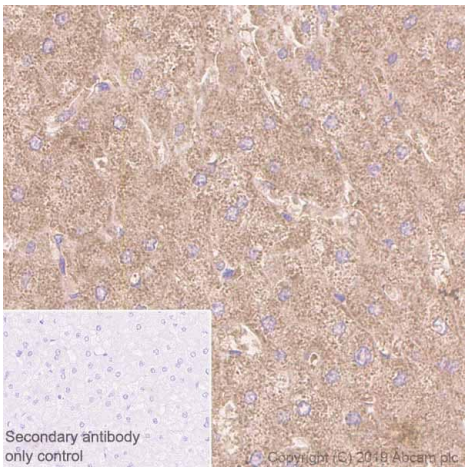
Secondary

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

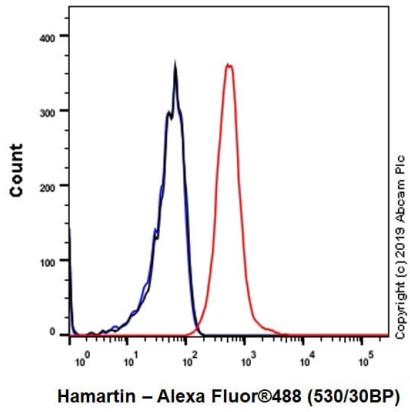
We are not sure about the nature of the extra band.

This data was developed using **ab40872**, the same antibody clone in a different buffer formulation.



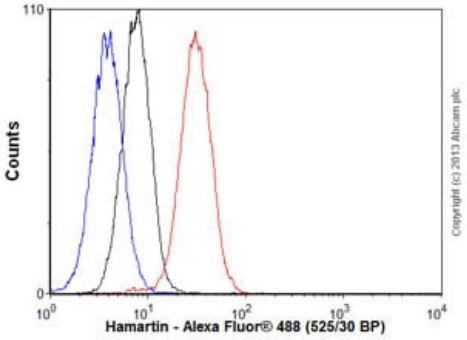
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hamartin antibody [EP318Y] - BSA and Azide free (ab247297)

This data was developed using **ab40872**, the same antibody clone in a different buffer formulation.
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.



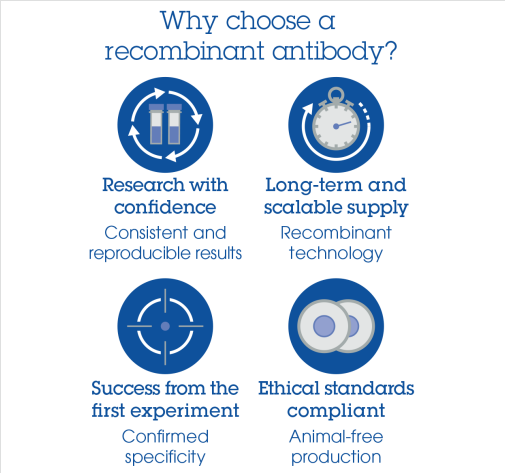
Flow Cytometry (Intracellular) - Anti-Hamartin antibody [EP318Y] - BSA and Azide free (ab247297)

This data was developed using **ab40872**, the same antibody clone in a different buffer formulation.Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Hamartin with Purified **ab40872** at 1/20 dilution (10µg/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).



Flow Cytometry (Intracellular) - Anti-Hamartin antibody [EP318Y] - BSA and Azide free (ab247297)

This data was developed using **ab40872**, the same antibody clone in a different buffer formulation.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.



Anti-Hamartin antibody [EP318Y] - BSA and Azide free (ab247297)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors