# abcam

## Product datasheet

## Anti-Tuberin antibody [Y320] ab32554





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#### Overview

**Product name** Anti-Tuberin antibody [Y320]

**Description** Rabbit monoclonal [Y320] to Tuberin

**Host species** Rabbit

Specificity This antibody recognises Tuberous sclerosis complex-2 (TCS2 also known as Tuberin. It is

predicted to detect isoform 2-4 based on sequence homology.

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

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide within Human Tuberin aa 1750-1850 (C terminal). The exact sequence is

proprietary.

Positive control WB: HAP1 and HeLa whole cell lysates. Rat and mouse brain tissue lysates; Flow Cyt (intra):

Jurkat cells; IHC: Rat and mouse liver tissue. Human lung carcinoma tissue.

**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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

pH: 7.2 Storage buffer

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

Protein A purified **Purity** 

**Clonality** Monoclonal

Clone number Y320
Isotype kgG

#### **Applications**

## The Abpromise guarantee

Our Abpromise guarantee covers the use of ab32554 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
Flow Cyt (Intra)		1/30.  ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100 dilution
WB	****(1)	1/1000 - 1/10000. Detects a band of approximately 220 kDa (predicted molecular weight: 200 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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**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

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.

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.

Sequence similarities

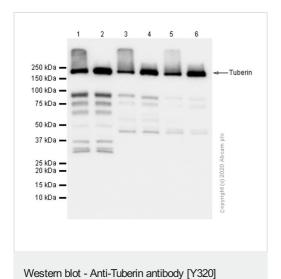
Contains 1 Rap-GAP domain.

Post-translational modifications

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.

#### **Images**

(ab32554)



**All lanes :** Anti-Tuberin antibody [Y320] (ab32554) at 1/5000 dilution (purified)

**Lane 1**: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate boiled

Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell)

whole cell lysate unboiled

Lane 3: Mouse brain lysate boiled

Lane 4: Mouse brain lysate unboiled

Lane 5: Rat brain lysate boiled

Lane 6: Rat brain lysate unboiled

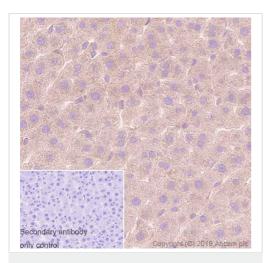
Lysates/proteins at 20 µg per lane.

## Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

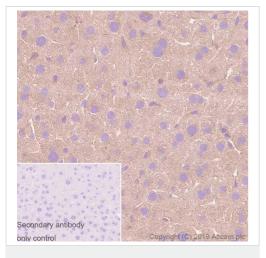
Predicted band size: 200 kDa Observed band size: 200 kDa

The unboiled lysate are recommended to be used in SDS-PAGE, in order to prevent membrane protein aggregation.



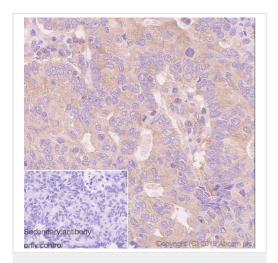
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tuberin antibody [Y320] (ab32554)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue sections labeling Tuberin with purified ab32554 at 1/100 dilution (2.97 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). 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.



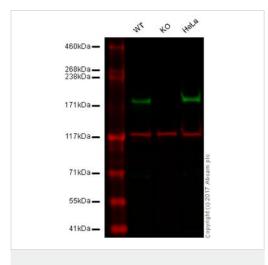
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tuberin antibody [Y320] (ab32554)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling Tuberin with purified ab32554 at 1/100 dilution (2.97 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). 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.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tuberin antibody [Y320] (ab32554)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue sections labeling Tuberin with purified ab32554 at 1/100 dilution (2.97 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). 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.



Western blot - Anti-Tuberin antibody [Y320] (ab32554)

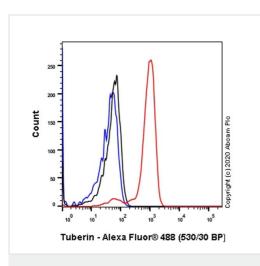
Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

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

Lane 3: HeLa whole cell lysate (20 µg)

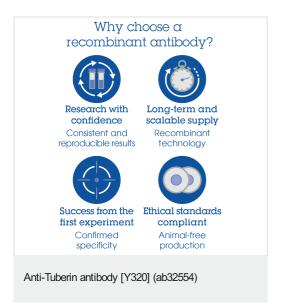
**Lanes 1 - 4:** Merged signal (red and green). Green - unpurified ab32554 observed at 180 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab32554 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. Ab32554 and <a href="mailto:ab18058">ab18058</a> (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 lgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-Tuberin antibody [Y320] (ab32554)

Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling Tuberin with purified ab32554 at 1/30 dilution (10µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluorr<sup>®</sup> 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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

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