# abcam

# Product datasheet

# Anti-PRAS40 antibody [EPR6263(2)] ab151719





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Overview

**Product name** Anti-PRAS40 antibody [EPR6263(2)]

Rabbit monoclonal [EPR6263(2)] to PRAS40 **Description** 

**Host species** Rabbit

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

Unsuitable for: ICC/IF

Reacts with: Mouse, Human Species reactivity

**Immunogen** Synthetic peptide within Human PRAS40 aa 150-250. The exact sequence is proprietary.

Database link: Q96B36

Positive control 293T, SHSY5Y, HeLa and NIH 3T3 cell lysates; Human liver tissue; 293T 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.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this

species. Please contact us for more information.

**Properties** 

**Form** Liquid

Storage instructions Shipped at 4°C. Store at -20°C.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

**Purity** Protein A purified

Clonality Monoclonal

Clone number EPR6263(2)

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise quarantee** covers the use of ab151719 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 - 1/10000. Detects a band of approximately 40 kDa (predicted molecular weight: 27 kDa).
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/10 - 1/100.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

**Application notes** 

Is unsuitable for ICC/IF.

#### **Target**

#### **Function**

Subunit of mTORC1, which regulates cell growth and survival in response to nutrient and hormonal signals. mTORC1 is activated in response to growth factors or amino-acids. Growth factorstimulated mTORC1 activation involves a AKT1-mediated phosphorylation of TSC1-TSC2, which leads to the activation of the RHEB GTPase that potently activates the protein kinase activity of mTORC1. Amino-acid-signaling to mTORC1 requires its relocalization to the lysosomes mediated by the Ragulator complex and the Rag GTPases. Activated mTORC1 up-regulates protein synthesis by phosphorylating key regulators of mRNA translation and ribosome synthesis. mTORC1 phosphorylates EIF4EBP1 and releases it from inhibiting the elongation initiation factor 4E (eiF4E). mTORC1 phosphorylates and activates S6K1 at 'Thr-389', which then promotes protein synthesis by phosphorylating PDCD4 and targeting it for degradation. Within mTORC1, AKT1S1 negatively regulates mTOR activity in a manner that is dependent on its phosphorylation state and binding to 14-3-3 proteins. Inhibits RHEB-GTP-dependent mTORC1 activation. Substrate for AKT1 phosphorylation, but can also be activated by AKT1-independent mechanisms. May also play a role in nerve growth factor-mediated neuroprotection.

#### Tissue specificity

Widely expressed with highest levels of expression in liver and heart. Expressed at higher levels in cancer cell lines (e.g. A549 and HeLa) than in normal cell lines (e.g. HEK293).

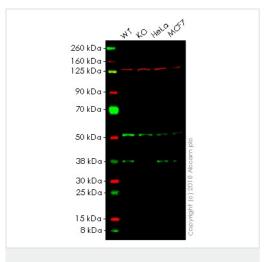
Phosphorylated by AKT1. Phosphorylation relieves inhibitory function on mTORC1.

Post-translational modifications

Cellular localization

Cytoplasm > cytosol. Found in the cytosolic fraction of the brain.

### **Images**



Western blot - Anti-PRAS40 antibody [EPR6263(2)] (ab151719)

**All lanes :** Anti-PRAS40 antibody [EPR6263(2)] (ab151719) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: AKT1S1 knockout HAP1 whole cell lysate

Lane 3 : HeLa whole cell lysate

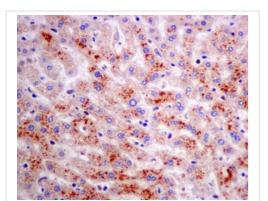
Lane 4 : MCF-7 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 27 kDa **Observed band size:** 40 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab151719 observed at 27 kDa. Red - loading control, <u>ab130007</u>, observed at 124 kDa.

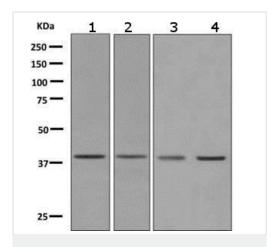
ab151719 was shown to recognize PRAS40 in wild-type HAP1 cells as signal was lost at the expected MW in AKT1S1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and AKT1S1 knockout samples were subjected to SDS-PAGE. Ab151719 and <a href="mailto:ab130007">ab130007</a> (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PRAS40 antibody
[EPR6263(2)] (ab151719)

Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling PRAS40 with ab151719 at 1/50 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-PRAS40 antibody [EPR6263(2)] (ab151719)

**All lanes :** Anti-PRAS40 antibody [EPR6263(2)] (ab151719) at 1/1000 dilution

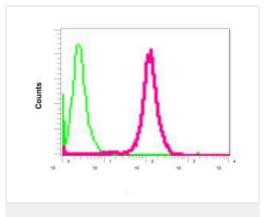
Lane 1: 293T cell lysate
Lane 2: SHSY5Y cell lysate
Lane 3: HeLa cell lysate
Lane 4: NIH 3T3 cell lysate

Lysates/proteins at 10 µg per lane.

# **Secondary**

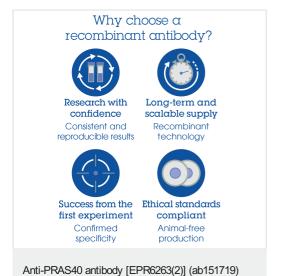
All lanes: Goat anti-rabbit HRP at 1/2000 dilution

**Predicted band size:** 27 kDa **Observed band size:** 40 kDa



Flow Cytometry (Intracellular) - Anti-PRAS40 antibody [EPR6263(2)] (ab151719)

Intracellular flow cytometric analysis of permeabilized 293T cells labeling PRAS40 with ab151719 at 1/10 dilution (red), compared to a nonspecific control antibody (green).



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