abcam

Product datasheet

Anti-RHEB antibody [EPR2971] ab92313



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Overview

Product name Anti-RHEB antibody [EPR2971]

Description Rabbit monoclonal [EPR2971] to RHEB

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF

Unsuitable for: IP

Species reactivity Reacts with: Human

Immunogen Synthetic peptide within Human RHEB aa 50-150. The exact sequence is proprietary.

(Peptide available as ab194764)

Positive control WB: Raji and SH-SY5Y lysates. IHC-P: human glioma and brain tissue. ICC/IF: Raji cells. Flow Cyt

(intra): Raji 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. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR2971

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab92313 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)		1/40. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100 - 1/1000
WB		1/2000 - 1/5000. Predicted molecular weight: 20 kDa.Can be blocked with Human RHEB peptide (ab194764) .
IHC-P	**** <u>(2)</u>	1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Heat mediated antigen retrieval and use of an HRP/AP polymerized secondary antibody recommended. See IHC antigen retrieval protocols For unpurified use at 1/100 - 1/250
ICC/IF		1/100 - 1/250.

Application notes

Is unsuitable for IP.

Target

Function	Stimulates the phosphorylation of S6K1 and EIF4EBP1 through activation of mTORC1 signaling.
	Activates the protein kinase activity of mTORC1. Has low intrinsic GTPase activity.

Tissue specificity Ubiquitous. Highest levels observed in skeletal and cardiac muscle.

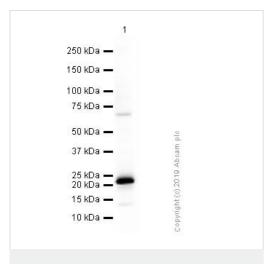
Sequence similaritiesBelongs to the small GTPase superfamily. Rheb family.

Post-translational modifications

Farnesylation is important for efficiently activating mTORC1-mediated signaling.

Cellular localization Cell membrane.

Images



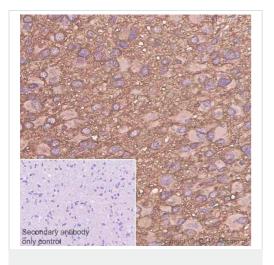
Western blot - Anti-RHEB antibody [EPR2971] (ab92313)

Anti-RHEB antibody [EPR2971] (ab92313) at 1/2000 dilution + Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysates at 15 µg

Secondary

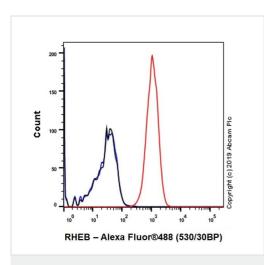
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 20 kDa



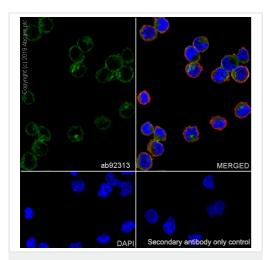
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RHEB antibody
[EPR2971] (ab92313)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human glioma tissue sections labeling RHEB with purified ab92313 at 1:500 dilution (0.73 µg/ml). Heat mediated antigen retrieval was performed 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.



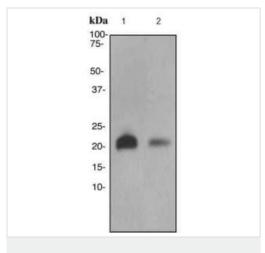
Flow Cytometry (Intracellular) - Anti-RHEB antibody [EPR2971] (ab92313)

Intracellular Flow Cytometry analysis of Raji (Human Burkitt's lymphoma B lymphocyte) cells labeling RHEB with purified ab92313 at 1/40 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor[®] 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).

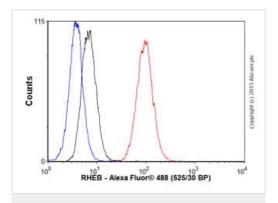


Immunocytochemistry/ Immunofluorescence - Anti-RHEB antibody [EPR2971] (ab92313)

Immunocytochemistry/ Immunofluorescence analysis of Raji (Human Burkitt's lymphoma B lymphocyte) cells labeling RHEB with purified ab92313 at 1:250 dilution (1.46 μ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 μ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-RHEB antibody [EPR2971] (ab92313)



Flow Cytometry (Intracellular) - Anti-RHEB antibody [EPR2971] (ab92313)

All lanes : Anti-RHEB antibody [EPR2971] (ab92313) at 1/2000 dilution

Lane 1 : Raji cell Isyate

Lane 2 : SH-SY5Y cell Isyate

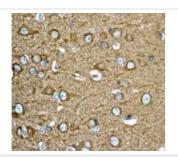
Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 20 kDa

Overlay histogram showing Raji cells stained with unpurifiedab92313 (red line). The cells were fixed with 4% paraformaldehyde (10 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 proteinprotein interactions followed by the antibody (ab92313, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor[®] 488 goat anti-rabbit lgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1µg/1x10⁶ 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 Raji cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RHEB antibody
[EPR2971] (ab92313)

Unpurified ab92313, at 1/100 dilution, staining RHEB in paraffinembedded Human brain tissue, by Immunohistochemistry.

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



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