abcam

ab316266

Anti-RHEB antibody [EPR25129-17] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR25129-17
Purification technique	Affinity purification Protein A
Concentration	1.025 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-

Notes	-

IP

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information Rheb

Function Small GTPase that acts as an allosteric activator of the canonical mTORC1 complex, an evolutionarily conserved central nutrient sensor that stimulates anabolic reactions and macromolecule biosynthesis to promote cellular biomass generation and growth (PubMed:12820960, PubMed:16631613, PubMed:21321084, PubMed:24648513). In response to nutrients, growth factors or amino acids, specifically activates the protein kinase activity of MTOR, the catalytic component of the mTORC1 complex: acts by causing a conformational change that allows the alignment of residues in the active site of MTOR, thereby enhancing the phosphorylation of ribosomal protein S6 kinase (RPS6KB1 and RPS6KB2) and EIF4EBP1 (4E-BP1) (By similarity). RHEB is also required for localization of the TSC-TBC complex to lysosomal membranes (By similarity). In response to starvation, RHEB is inactivated by the TSC-TBC complex, preventing activation of mTORC1 (By similarity). Has low intrinsic GTPase activity (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

ab316266 is the carrirer-free version of ab316265.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	RHEB also known as Ras homolog enriched in brain is a small GTPase with a molecular mass of approximately 21 kDa. It is a member of the Ras superfamily and plays a role in the regulation of cell growth and proliferation. RHEB is widely expressed with concentrations found in the brain skeletal muscle and various other tissues. It functions as a molecular switch cycling between active GTP-bound and inactive GDP-bound states influencing signaling pathways linked to growth and metabolism.
Biological function summary	RHEB interacts with several components within the mTORC1 complex a major regulator of cell growth and autophagy. This protein positively regulates the mTORC1 pathway by activating mTOR leading to protein synthesis and cell growth when nutrients such as amino acids and energy are available. RHEB's regulation of mTORC1 is influenced by upstream modulation through the TSC1/2 complex acting as a sensor for cellular energy levels and growth signals. The role of RHEB in these regulatory mechanisms highlights its fundamental importance in cellular metabolic processes.

Pathways	RHEB plays a significant role in the mechanistic target of rapamycin (mTOR) signaling pathway which is important for regulating protein synthesis and cellular growth. RHEB acts downstream of the TSC1/2 complex with TSC2 acting as a GTPase-activating protein for RHEB. This interaction points RHEB as an essential regulator of mTORC1 activation. In connection with other proteins RHEB functions alongside mTOR and TSC1/2 to mediate responses to cellular nutrient status thereby adjusting growth and proliferation accordingly.
Associated diseases and disorders	Mutations or dysregulation of RHEB have been linked to tuberous sclerosis complex (TSC) and cancer. In TSC where aberrant signaling occurs through the mTOR pathway RHEB's activity influences the mTORC1 pathway in cells lacking functional TSC1 or TSC2 leading to benign tumors. Additionally RHEB overexpression has been implicated in certain cancers due to its capacity to enhance cell proliferation via mTORC1. The interaction of RHEB with mTOR highlights its role in disease states where cell growth control is altered suggesting the potential for targeted therapies in treating these conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

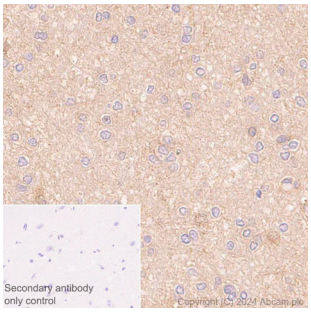
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

14 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling RHEB with ab316265 at 1/500 (0.98 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

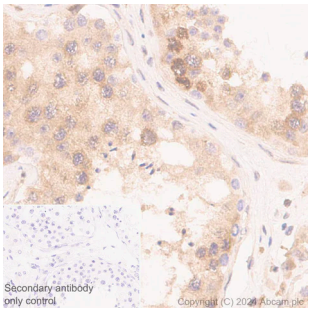
Positive staining in human cerebrum (PMID: 27512609).
The section was incubated with ab316265 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling RHEB with ab316265 at 1/500 (0.98 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

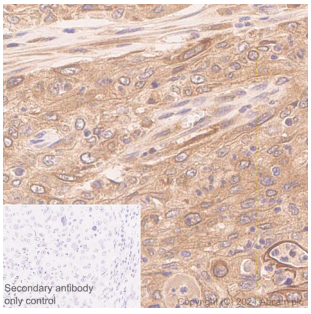
Positive staining in human testis.
The section was incubated with ab316265 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human ovarian cancer tissue labeling RHEB with ab316265 at 1/500 (0.98 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™

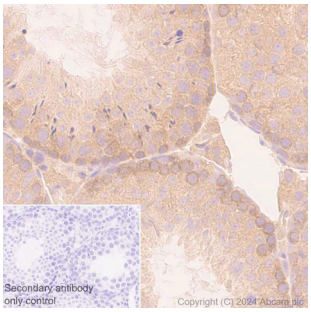
Polymer Refine Detection).

Positive staining in human ovarian cancer.
The section was incubated with ab316265 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

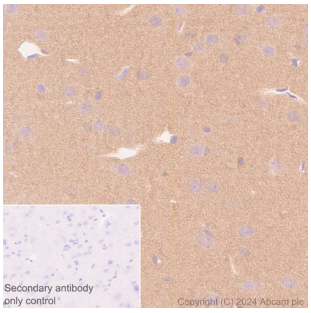
Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling RHEB with ab316265 at 1/5000 (0.098 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in mouse testis.
The section was incubated with ab316265 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

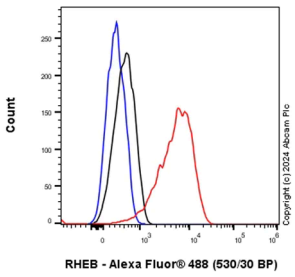
Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling RHEB with ab316265 at 1/5000 (0.098 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in rat cerebrum (PMID:8206940).
The section was incubated with ab316265 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

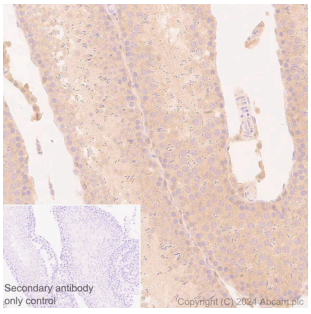


Flow Cytometry (Intracellular) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Neuro-2a (mouse neuroblastoma neuroblast) cells labelling RHEB with ab316265 at 1/50 dilution (1 ug)/Red compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

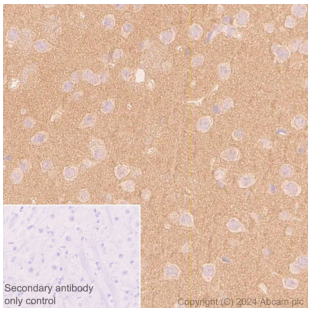
Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling RHEB with ab316265 at 1/5000 (0.098 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in rat testis (PMID: 8206940).
The section was incubated with ab316265 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling RHEB with ab316265 at 1/5000 (0.098 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

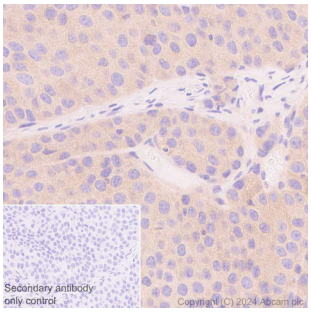
Positive staining in mouse cerebrum.
The section was incubated with ab316265 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

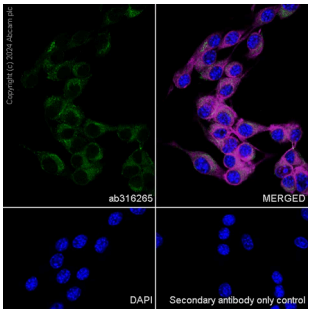
Immunohistochemical analysis of paraffin-embedded Mouse breast cancer tissue labeling RHEB with ab316265 at 1/5000 (0.098 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in mouse breast cancer tissue.
The section was incubated with ab316265 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

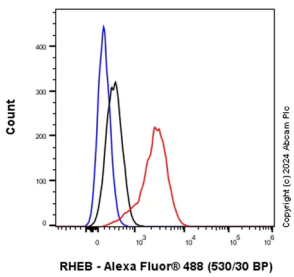
This data was developed using ab316265, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized Neuro-2a (mouse neuroblastoma neuroblast) cells labelling RHEB with ab316265 at 1/50 (9.8 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green).

Confocal image showing cytoplasmic staining in Neuro-2a cell line.
Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.

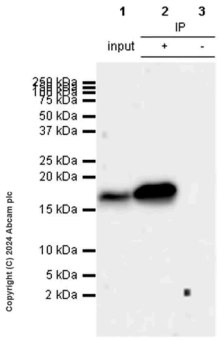


Flow Cytometry (Intracellular) - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling RHEB with ab316265 at 1/50 dilution (1 ug)/Red compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

RHEB was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) whole cell lysate with ab316265 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab316265 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 2: ab316265 IP in NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab316265 in NIH/3T3 whole cell lysate

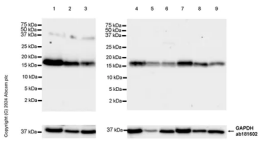
All lanes:
Immunoprecipitation - Anti-RHEB antibody [EPR25129-17] (ab316265) at 1/30 dilution

All lanes:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 119s



Western blot - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

RHEB Western blot staining using rabbit Anti-RHEB antibody

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

Lanes 1-3 are applied with Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 and Lanes 4-9 are applied with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000.

The identity of the bands around 37 kDa (in lanes 1-3) are unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

Exposure time: Lane 1-3: 37 seconds; Lane 3-7: 81 seconds
All lanes:
Western blot - Anti-RHEB antibody [EPR25129-17] (ab316265) at 1/1000 dilution

Lane 1:
Human brain tissue lysate at 20 µg

Lane 2:
Human testis tissue lysate at 20 µg

Lane 3:
Human liver tissue lysate at 20 µg

Lane 4:
Mouse brain tissue lysate at 20 µg

Lane 5:
Mouse testis tissue lysate at 20 µg

Lane 6:
Mouse liver tissue lysate at 20 µg

Lane 7:
Rat brain tissue lysate at 20 µg

Lane 8:
Rat testis tissue lysate at 20 µg

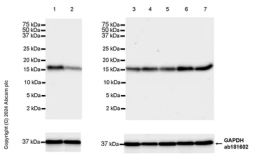
Lane 9:
Rat liver tissue lysate at 20 µg

Secondary

Lanes 1 - 3:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG (Merck DC03L) at 1/2000 dilution

Lanes 4 - 9:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 16 kDa, 36 kDa



Western blot - Anti-RHEB antibody [EPR25129-17] - BSA and Azide free (ab316266)

RHEB Western blot staining using rabbit Anti-RHEB antibody

This data was developed using ab316265, the same antibody clone in a different buffer formulation.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

Exposure time: Lane 1-2: 81 seconds; Lane 3-7: 59 seconds

All lanes:
Western blot - Anti-RHEB antibody [EPR25129-17] (ab316265) at 1/1000 dilution

Lane 1:
NIH/3T3 (mouse embryonic fibroblast) transfected with scrambled siRNA control whole cell lysate at 20 µg

Lane 2:
NIH/3T3 transfected with siRNA specifically targeting RHEB whole cell lysate at 20 µg

Lane 3:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 4:
Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate at 20 µg

Lane 5:
C2C12 (mouse myoblasts myoblast) whole cell lysate at 20 µg

Lane 6:
SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate at 20 µg

Lane 7:
PC-12 (rat adrenal gland pheochromocytoma cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 16 kDa, 36 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.