abcam

ab203558

Anti-S6K1 antibody [E343] - BSA and Azide free

Recombinant | RabMAb | KO Validated

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	E343
Purification technique	Affinity purification Protein A
Specificity	This antibody detects both alpha I and alpha II isoforms.
Concentration	1.007 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	For Rat and Mouse samples 1/500 dilution has only been tried. We have not tested if similarly to Human samples a lot higher dilutions can be used.

Expected

Species	Mouse
Dilution info	-
Notes	For Rat and Mouse samples 1/500 dilution has only been tried. We have not tested if similarly to Human samples a lot higher dilutions can be used.

Species	Rat
Dilution info	-
Notes	For Rat and Mouse samples 1/500 dilution has only been tried. We have not tested if similarly to Human samples a lot higher dilutions can be used.

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

Predicted

Species	Rat
Dilution info	-

Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information RPS6KB1

Function	Serine/threonine-protein kinase that acts downstream of mTOR signaling in response to growth factors and nutrients to promote cell proliferation, cell growth and cell cycle progression (PubMed:11500364, PubMed:12801526, PubMed:14673156, PubMed:15071500, PubMed:15341740, PubMed:16286006, PubMed:17052453, PubMed:17053147, PubMed:17936702, PubMed:18952604, PubMed:19085255, PubMed:19720745, PubMed:19935711, PubMed:19995915, PubMed:22017876, PubMed:23429703, PubMed:28178239). Regulates protein synthesis through phosphorylation of EIF4B, RPS6 and EEF2K, and contributes to cell survival by repressing the pro-apoptotic function of BAD (PubMed:11500364, PubMed:12801526, PubMed:14673156, PubMed:15071500, PubMed:15341740, PubMed:16286006, PubMed:17052453, PubMed:17053147, PubMed:17936702, PubMed:18952604, PubMed:19085255, PubMed:19720745, PubMed:19935711, PubMed:19995915, PubMed:22017876, PubMed:23429703, PubMed:28178239). Under conditions of nutrient depletion, the inactive form associates with the EIF3 translation initiation complex (PubMed:16286006). Upon mitogenic stimulation, phosphorylation by the mechanistic target of rapamycin complex 1 (mTORC1)

leads to dissociation from the EIF3 complex and activation (PubMed:16286006). The active form then phosphorylates and activates several substrates in the pre-initiation complex, including the EIF2B complex and the cap-binding complex component EIF4B (PubMed:16286006). Also controls translation initiation by phosphorylating a negative regulator of EIF4A, PDCD4, targeting it for ubiquitination and subsequent proteolysis (PubMed:17053147). Promotes initiation of the pioneer round of protein synthesis by phosphorylating POLDIP3/SKAR (PubMed:15341740). In response to IGF1, activates translation elongation by phosphorylating EEF2 kinase (EEF2K), which leads to its inhibition and thus activation of EEF2 (PubMed:11500364). Also plays a role in feedback regulation of mTORC2 by mTORC1 by phosphorylating MAPKAP1/SIN1, MTOR and RICTOR, resulting in the inhibition of mTORC2 and AKT1 signaling (PubMed:15899889, PubMed:19720745, PubMed:19935711, PubMed:19995915). Also involved in feedback regulation of mTORC1 and mTORC2 by phosphorylating DEPTOR (PubMed:22017876). Mediates cell survival by phosphorylating the pro-apoptotic protein BAD and suppressing its pro-apoptotic function (By similarity). Phosphorylates mitochondrial URI1 leading to dissociation of a URI1-PPP1CC complex (PubMed:17936702). The free mitochondrial PPP1CC can then dephosphorylate RPS6KB1 at Thr-412, which is proposed to be a negative feedback mechanism for the RPS6KB1 anti-apoptotic function (PubMed:17936702). Mediates TNF-induced insulin resistance by phosphorylating IRS1 at multiple serine residues, resulting in accelerated degradation of IRS1 (PubMed:18952604). In cells lacking functional TSC1-2 complex, constitutively phosphorylates and inhibits GSK3B (PubMed:17052453). May be involved in cytoskeletal rearrangement through binding to neurabin (By similarity). Phosphorylates and activates the pyrimidine biosynthesis enzyme CAD, downstream of MTOR (PubMed:23429703). Following activation by mTORC1, phosphorylates EPRS and thereby plays a key role in fatty acid uptake by adipocytes and also most probably in interferon-gamma-induced translation inhibition (PubMed:28178239).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab203558 is the carrier-free version of ab32529.

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	S6K1 also known as p70S6K is a serine/threonine kinase involved in protein synthesis. The protein has a molecular weight of approximately 70 kDa. Scientists often examine the presence of S6K1 in various tissues as it is expressed ubiquitously in eukaryotic cells. This kinase plays an instrumental role in the regulation of cell growth proliferation and survival by phosphorylating ribosomal protein S6 and other targets. Researchers explore various cellular contexts where S6K1 activity is implicated due to its widespread expression and function.
Biological function summary	S6K1 functions as a downstream effector of the mammalian target of rapamycin (mTOR) complex specifically mTORC1. S6K1 interacts with several protein complexes enhancing its regulatory capacity in cellular activities. It modulates protein synthesis by phosphorylating substrates involved in translation. By controlling these processes S6K1 aids in cell size regulation and energy metabolism which are critical for maintaining cellular homeostasis and adaptation to nutrient availability.
Pathways	S6K1 plays an essential role in the mTOR signaling pathway a central regulator of cell growth and metabolism. S6K1 functions in close connection with proteins such as mTOR and Raptor within this pathway. Additionally it is involved in the insulin signaling pathway where it works with proteins like insulin receptor substrate (IRS). Both pathways highlight S6K1's role in nutrient sensing and response emphasizing its significance in energy and protein homeostasis.
Associated diseases and disorders	S6K1 is associated with conditions such as cancer and type 2 diabetes. Dysregulation of S6K1 activity can lead to abnormal cell proliferation contributing to oncogenesis in various cancer types. Specifically altered signaling through

pathways involving mTOR and Akt proteins connects S6K1 to cancer progression. In type 2 diabetes S6K1 affects insulin sensitivity where overactive S6K1 signaling leads to insulin resistance. Here interactions with insulin receptor substrate proteins highlight its influence on metabolic disorders underlining its critical role in disease pathology.

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

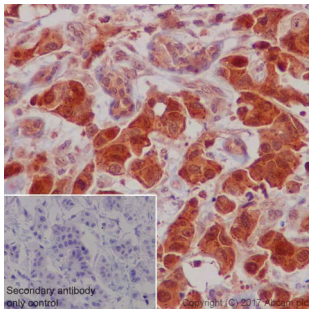
We do not recommend this combination. It is not covered by our product promise.

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.

18 product images

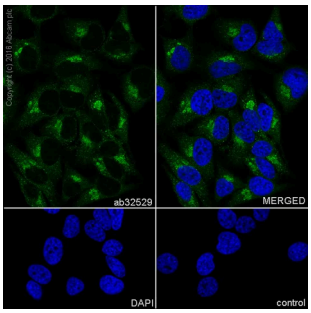


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-S6K1 antibody

Immunohistochemical analysis of Human breast cancer tissue labeling S6K1 with ab32529 at 1/500 dilution (4.4 µg/mL). The secondary antibody used was ImmunoHistoProbe one step HRP Polymer (ready to use). Secondary antibody only control-PBS instead of the primary antibody. Antigen retrieval was heat mediated using ab93684 (Tris/EDTA buffer, pH 9.0). The tissue was counterstained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).

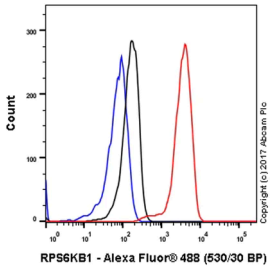


Immunocytochemistry/ Immunofluorescence - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-S6K1 antibody

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling S6K1 with ab32529 at a dilution of 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. ab150077 at 1/1000 was used as the secondary antibody. Nuclei were counterstained with DAPI (blue).
Confocal image showing cytoplamic staining on HeLa cell line.

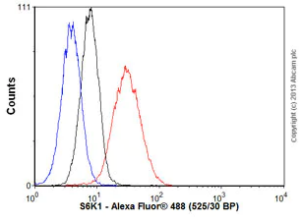
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Flow Cytometry (Intracellular) - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Flow Cytometry (Intracellular) staining using rabbit Anti-S6K1 antibody

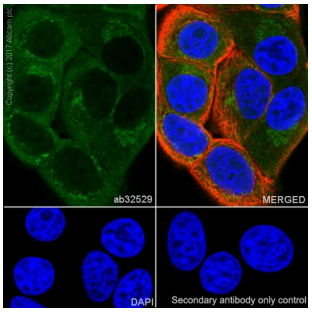
Intracellular Flow Cytometry analysis of 293T (Human embryonic kidney epithelial cell) cells labelling with ab32529 (purified) at 1/2200 dilution (1 µg/mL) (red). Cells were fixed with 4% paraformaldehyde . Goat anti rabbit IgG (Alexa Fluorr® 488, ab150077) was used as the secondary antibody at 1/2000 dilution. Isotype control - 90% methanol . Unlabeled control - Rabbit monoclonal IgG (ab172730) / Black.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Flow Cytometry (Intracellular) - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Flow Cytometry (Intracellular) staining using rabbit Anti-S6K1 antibody

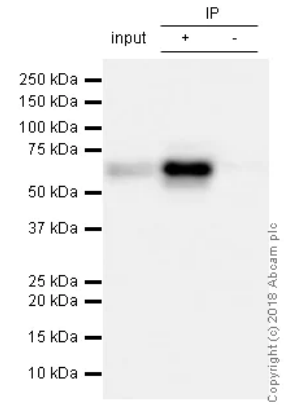
Overlay histogram showing HeLa cells stained with ab32529 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32529, 1/1000 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 data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Immunocytochemistry/ Immunofluorescence - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-S6K1 antibody

Immunocytochemistry/Immunofluorescence analysis of MCF 7 (Human breast adenocarcinoma epithelial cell) labeling S6K1 with ab32529 at a dilution of 1:200, 11.1 ug/ml. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. A dilution of 1/1000 (2µg/ml) was used for the secondary antibodyGoat anti rabbit IgG (Alexa Fluor® 488, ab150077). The cells were co-stained at 1:200 dilution, 2.5µg/ml with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) . Nuclei counterstained with DAPI (blue). Control: 1:1000 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Immunoprecipitation - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

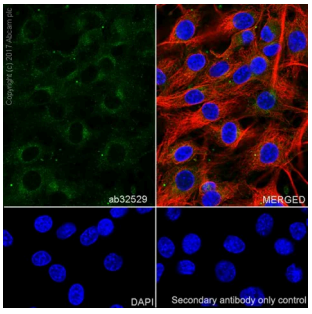
Lane 1: HEK293T (Human embryonic kidney epithelial cell) whole cell lysate, 10µg
Lane 2: HEK293T whole cell lysate, 10µg and ab32529, 2µg
Lane 3: HEK293T cell lysate, 350µg and rabbit IgG (ab172730) , 2µg

Purified ab32529 immunoprecipitating S6K1 in HEK293T cell lysates. Primary antibody was used at a 1:500 dilution (4.4 µg/ml). For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

Blocking and diluting buffer used: 5% NFDM/TBST.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).

All lanes:
Immunoprecipitation - Anti-S6K1 antibody [E343] (ab32529)

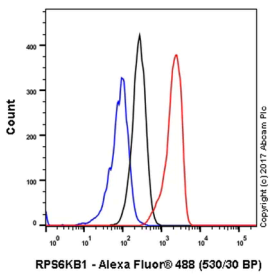
Predicted band size: 59 kDa



Immunocytochemistry/ Immunofluorescence - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-S6K1 antibody

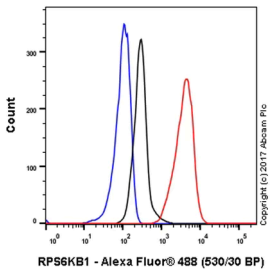
Immunocytochemistry/Immunofluorescence analysis of C6 cells (Rat glial tumor glial cell) labelling S6K1 with ab32529 at a dilution of 1:200, 11.1 µg/ml. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. A 1:1000 dilution (2µg/ml) was used for the secondary antibody Goat anti rabbit IgG (Alexa Fluor® 488, ab150077). The cells were co-stained with 1:200, 2.5µg/ml with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594). Nuclei counterstained with DAPI (blue). Control: 1:1000 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Flow Cytometry (Intracellular) - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Flow Cytometry (Intracellular) staining using rabbit Anti-S6K1 antibody

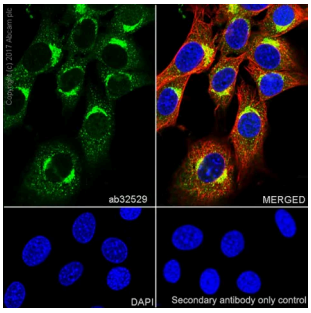
Intracellular Flow Cytometry analysis of C6 (Rat glial tumor glial cell) cells labelling with ab32529 (purified) at 1/2200 dilution (1 µg/mL) (red). Cells were fixed with 4% paraformaldehyde . Goat anti rabbit IgG (Alexa Fluorr® 488, ab150077) was used as the secondary antibody at 1/2000 dilution. Isotype control - 90% methanol . Unlabeled control - Rabbit monoclonal IgG (ab172730) / Black.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Flow Cytometry (Intracellular) - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Flow Cytometry (Intracellular) staining using rabbit Anti-S6K1 antibody

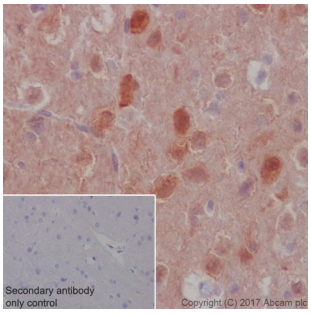
Intracellular Flow Cytometry analysis of Neuro-2a (Mouse neuroblastoma neuroblast) cells labelling with ab32529 (purified) at 1/2200 dilution (1 µg/mL) (red). Cells were fixed with 4% paraformaldehyde . Goat anti rabbit IgG (Alexa Fluorr® 488, ab150077) was used as the secondary antibody at 1/2000 dilution. Isotype control - 90% methanol . Unlabeled control - Rabbit monoclonal IgG (ab172730) / Black.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Immunocytochemistry/ Immunofluorescence - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-S6K1 antibody

Immunocytochemistry/Immunofluorescence analysis of NIH/3T3 (Mouse embryonic fibroblast) labelling with ab32529 at a dilution of 1:200, 11.1 µg/ml. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. A 1:1000 dilution (2µg/ml) was used for the secondary antibody Goat anti rabbit IgG (Alexa Fluor® 488, ab150077). The cells were co-stained at 1:200 dilution, 2.5µg/ml with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594). Nuclei counterstained with DAPI (blue). Control: 1:1000 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).

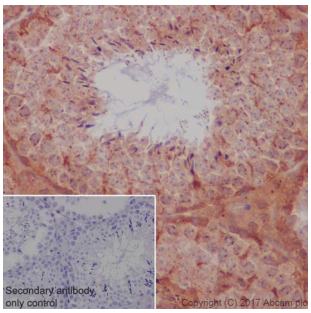


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-S6K1 antibody

Immunohistochemical analysis of rat brain tissue labeling S6K1 with ab32529 at 1/500 dilution (4.4 µg/mL). The secondary antibody used was ImmunoHistoProbe one step HRP Polymer (ready to use). Secondary antibody only control-PBS instead of the primary antibody. Antigen retrieval was

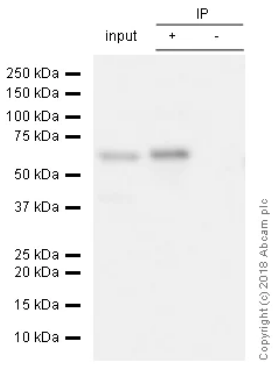
heat mediated using ab93684 (Tris/EDTA buffer, pH 9.0). The tissue was counterstained with Hematoxylin.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-S6K1 antibody

Immunohistochemical analysis of mouse testis tissue labeling S6K1 with ab32529 at 1/500 dilution (4.4 µg/mL). The secondary antibody used was ImmunoHistoProbe one step HRP Polymer (ready to use). Secondary antibody only control-PBS instead of the primary antibody. Antigen retrieval was heat mediated using ab93684 (Tris/EDTA buffer, pH 9.0). The tissue was counterstained with Hematoxylin.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).



Immunoprecipitation - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

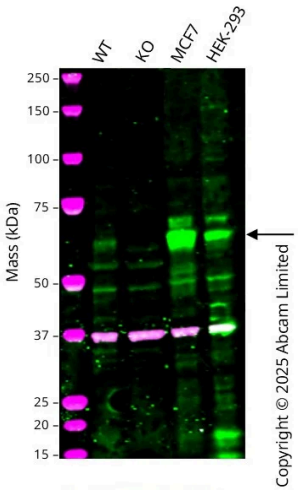
Lane 1: Neuro2a (Mouse neuroblastoma neuroblast) whole cell lysate, 10µg
Lane 2: Neuro2a whole cell lysate 350µg and ab32529, 2µg
Lane 3: Neuro2a cell lysate, 350µg and rabbit IgG (ab172730), 2µg

Purified ab32529 immunoprecipitating S6K1 in HEK293T cell lysates. Primary antibody was used at a 1:500 dilution (4.4 µg/ml). For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.
Blocking and diluting buffer used: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).

All lanes:
Immunoprecipitation - Anti-S6K1 antibody [E343] (ab32529)

Predicted band size: 59 kDa



Western blot - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Western blot staining using rabbit Anti-S6K1 antibody

This data was developed using ab32529, the same antibody clone in a different buffer formulation. Western blot: Rabbit Monoclonal[E343] to S6K1 ab32529 staining at 1/10000 dilution, shown in green; Mouse anti GAPDH (ab8245) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 59 kDa in Wild-type U-87 MG cell lysates with no signal observed at this size in RPS6KB1 knockout U-87 MG cell line (ab326023). To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - Anti-S6K1 antibody [E343] (ab32529) at 1/10000 dilution

Lane 1:
Wild-type U-87 MG at 20 µg

Lane 2:
Western blot - Human RPS6KB1 knockout U-87 MG cell line (ab326023) at 20 µg

Lane 2:
RPS6KB1 knockout U-87 MG at 20 µg

Lane 3:
MCF7 at 20 µg

Lane 4:
HEK-293 at 20 µg

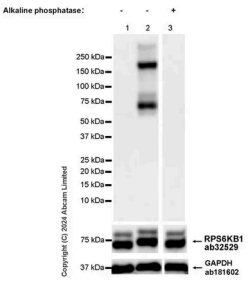
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 59 kDa

Observed band size: 59 kDa



Western blot - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Western blot staining using rabbit Anti-S6K1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The identity of the higher MW band at approximately 150 kDa (in lane 2) is unknown.

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

In Western blot, Anti-RPS6KB1 antibody [E343] (ab32529) staining at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).
All lanes:
Western blot - Anti-S6K1 (phospho T389) antibody [EPR24766-9] (ab323272) at 1/1000 dilution

Lane 1:
Untreated HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 50 µg

Lane 2:
Hela treated with 100nM Calycin A for 30min whole cell lysate at 50 µg

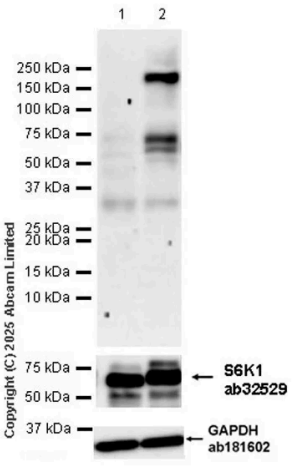
Lane 3:
Hela treated with 100nM Calycin A for 30min whole cell lysate (alkaline phosphatase treated membrane) at 50 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 60-80 kDa, 36 kDa

Exposure time: 37s



Western blot - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Western blot staining using rabbit Anti-S6K1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

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

In Western blot, Anti-RPS6KB1 antibody [E343] - Total protein control (ab32529) staining at 1/1000 dilution.

The identity of the bands at approximately 150 kDa (in lane 2) and at approximately 30 kDa (in lanes 1-2) are unknown.

All lanes:
Western blot - Anti-S6K1 (phospho T389) antibody [EPR24766-9] (ab323272) at 1/1000 dilution

Lane 1:
Untreated NIH/3T3 (mouse embryonic fibroblast) starved overnight, whole cell lysate at 20 µg

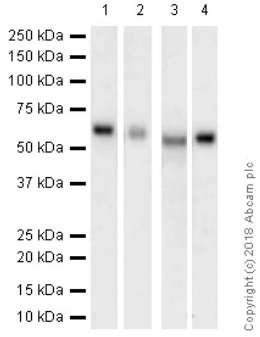
Lane 2:
NIH/3T3 starved overnight, then treated with 100nM Calyculin A for 30min whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/200000 dilution

Observed band size: 60-80 kDa

Exposure time: 180s



Western blot - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Western blot staining using rabbit Anti-S6K1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).
Blocking and diluting buffer used: 5% NFDM/TBST.

All lanes:
Western blot - Anti-S6K1 antibody [E343] (ab32529) at 0.004 µg/mL

Lane 1:
Neuro2a (Mouse neuroblastoma neuroblast) whole cell lysate at 20 µg

Lane 2:
Mouse cerebellum lysate at 20 µg

Lane 3:
C6 (Rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 4:

Rat cerebellum at 20 µg

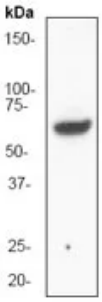
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 59 kDa

Observed band size: 70 kDa

Exposure time: 3min



Western blot - Anti-S6K1 antibody [E343] - BSA and Azide free (ab203558)

S6K1 Western blot staining of 293T cell lysate using rabbit Anti-S6K1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32529).
All lanes:
Western blot - Anti-S6K1 antibody [E343] (ab32529) at 1/10000 dilution

All lanes:
293T cell lysate

Predicted band size: 59 kDa

Observed band size: 70 kDa

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