abcam

Product datasheet

Anti-S6K1 antibody [E175] ab32359

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-S6K1 antibody [E175]
Description	Rabbit monoclonal [E175] to S6K1
Host species	Rabbit
Specificity	This antibody may detect both phosphorylated and non-phosphorylated forms of p70 S6 Kinase.
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Cow
Immunogen	Synthetic peptide within Human S6K1. The exact sequence is proprietary.
Positive control	Jurkat cell lysate; human breast carcinoma WB: C-Myc/DDK tagged human S6K1 recombinant protein, C-Myc/DDK tagged human S6K2 recombinant protein.
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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Protein A purified

Clonality	Monoclonal
Clone number	E175
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32359 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)	1/1000 - 1/5000. Detects a band of approximately 70 kDa (predicted molecular weight: 59 kDa).
IHC-P	★★★☆☆ (2)	1/50. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Application notes Is unsuitable for Flow Cyt or ICC/IF.

Target

Function Acts to integrate nutrient and growth factor signals in regulation of protein synthesis, cell proliferation, cell growth, cell cycle progression and cell survival. Downstream effector of the mTOR signaling pathway. Phosphorylates specifically ribosomal protein S6 in response to insulin or several classes of mitogens. During translation initiation, the inactive form associatess with the eIF-3 complex under conditions of nutrient depletion. Mitogenic stimulation leads to phosphorylation and dissociation from the eIF-3 complex and the free activated form can phosphorylate other translational targets including EIF4B. Promotes protein synthesis by phosphorylating PDCD4 at 'Ser-67' and targeting it for degradation. Phosphorylates RICTOR leading to regulation of mammalian target of rapamycin complex 2 (mTORC2) signaling; probably phosphorylates RICTOR at 'Thr-1135'. Phosphorylates IRS1 at multiple serine residues coupled with insulin resistance; probably phosphorylates IRS1 at 'Ser-270'. Required for TNF-alpha induced IRS-1 degradation. Phosphorylates EEF2K in response to IGF1 and inhibits EEF2K activity. Phosphorylates BAD at 'Ser-99' in response to IGF1 leading to BAD inactivation and inhibition of BAD-induced apoptosis. Phosphorylates mitochondrial RMP leading to dissociation of a RMP:PPP1CC complex; probably phosphorylates RMP at 'Ser-99'. The free mitochondrial PPP1CC can dephosphorylate RPS6KB1 at Thr-412 which is proposed to be a negative feed back mechanism for the RPS6KB1 antiapoptotic function. Phosphorylates GSK3B at 'Ser-9' under conditions leading to loss of the TSC1-TSC2 complex. Phosphorylates POLDIP3.

Tissue specificity Widely expressed.

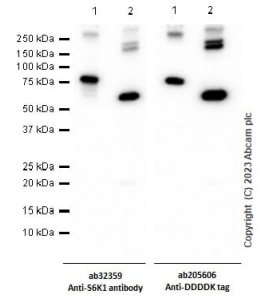
Sequence similarities Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. S6 kinase subfamily.
Contains 1 AGC-kinase C-terminal domain.
Contains 1 protein kinase domain.

Domain The autoinhibitory domain is believed to block phosphorylation within the AGC-kinase C-terminal domain and the activation loop.
The TOS (TOR signaling) motif is essential for activation by mTORC1.

Post-translational Phosphorylation at Thr-412 is regulated by mTORC1. The phosphorylation at this site is

modifications	maintained by an agonist-dependent autophosphorylation mechanism.
Cellular localization	Cytoplasm; Nucleus. Cytoplasm and Cell junction > synapse > synaptosome. Mitochondrion outer membrane.

Images



Western blot - Anti-S6K1 antibody [E175] (ab32359)

All lanes : Anti-S6K1 antibody [E175] (ab32359) at 1/1000 dilution

Lane 1 : C-Myc/DDK tagged human S6K1 recombinant protein 10ng

Lane 2 : C-Myc/DDK tagged human S6K2 recombinant protein 10ng

Secondary

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

Predicted band size: 59 kDa

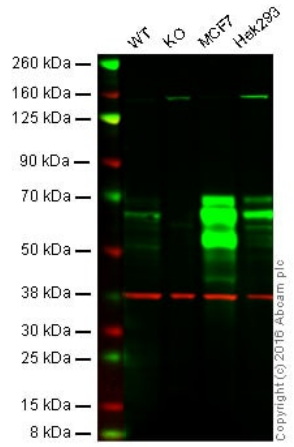
Observed band size: 60,75 kDa

Exposure time: 10 seconds

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

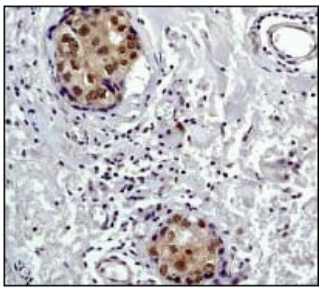
ab205606 was also used at 1/1000 dilution to detect C-Myc/DDK tagged human S6K1 and S6K2 recombinant proteins shown on the right panel.

ab32539 showed cross reactivity with S6K2 in recombinant protein testing. S6K1 and S6K2 have different observed band size in endogenous and recombinant protein tests.



Western blot - Anti-S6K1 antibody [E175] (ab32359)

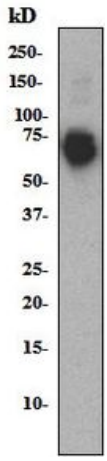
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: S6K1 knockout HAP1 cell lysate (20 µg)
Lane 3: MCF7 cell lysate (20 µg)
Lane 4: HEK293 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab32359 observed at 68 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab32359 was shown to recognize S6K1 when S6K1 knockout samples were used, along with additional cross-reactive bands. Wild-type and S6K1 knockout samples were subjected to SDS-PAGE. ab32359 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S6K1 antibody [E175] (ab32359)

Paraffin-embedded human breast carcinoma
ab32359 at 1/50 dilution

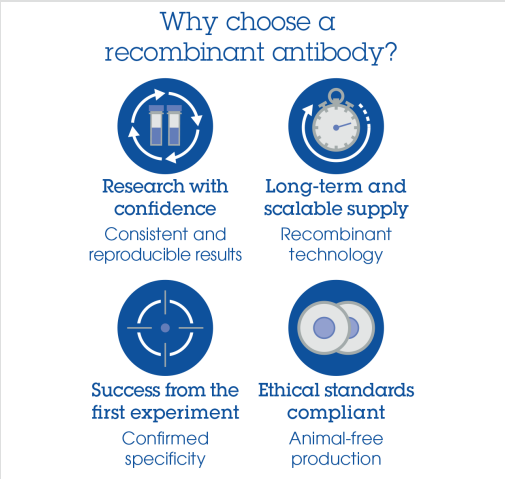
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-S6K1 antibody [E175] (ab32359)

Anti-S6K1 antibody [E175] (ab32359) + HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Predicted band size: 59 kDa



Anti-S6K1 antibody [E175] (ab32359)

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