

Product Datasheet

p70 S6 Kinase/S6K [p Thr389/412] Antibody - BSA Free NB600-1049-0.1mg

Unit Size: 0.1 mg

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB600-1049-0.1mg

p70 S6 Kinase/S6K [p Thr389/412] Antibody - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Affinity purified
Buffer	PBS (pH 7.4), 150 mM EDTA, 50% glycerol

Product Description

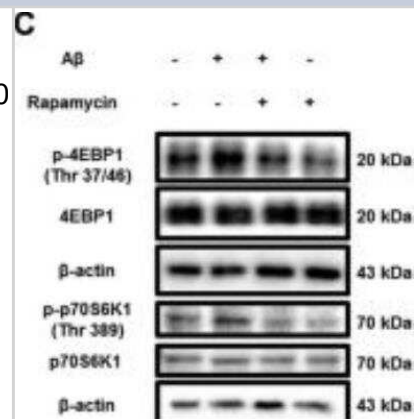
Description	This product is manufactured by Abcam and distributed by Novus Biologicals. (Abcam Catalog Number: ab2571)
Host	Rabbit
Gene ID	6198
Gene Symbol	RPS6KB1
Species	Human, Mouse, Rat
Specificity/Sensitivity	p70 S6 Kinase/S6K [p Thr389/412] Antibody detects endogenous levels of p70 S6 Kinase only when phosphorylated at Threonine 389/412
Immunogen	Synthetic phospho-peptide surrounding amino acids Thr389/412 of human p70 S6 Kinase/S6K

Product Application Details

Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:500 - 1:2000 , Immunohistochemistry 1:50 - 1:200 , Immunocytochemistry/ Immunofluorescence 1:100 - 1:500, Immunohistochemistry-Paraffin
Application Notes	Use in Immunohistochemistry-Paraffin reported in scientific literature (PMID:32339950).

Images

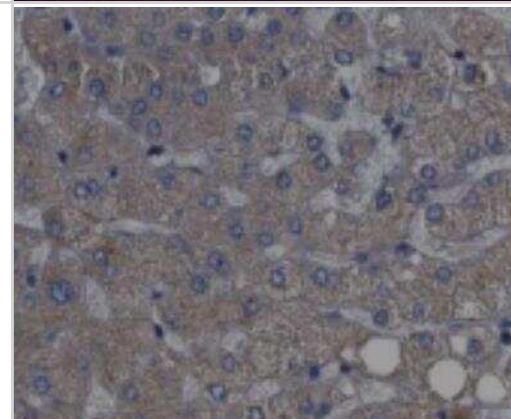
Western Blot: p70 S6 Kinase/S6K [p Thr389/412] Antibody [NB600-1049] - Abeta facilitates HIF1alpha synthesis and autophagy inhibition via mTOR activation. Cells were incubated with rapamycin (10 nM) for 30 min prior to Abeta treatment for 24 h. Phosphorylation of 4EBP1 (Thr 37/46) and 4EBP1, phosphorylation of p70S6K1 (Thr 389), HIF1alpha and beta-actin were analyzed by western blot. n = 6. Image collected and cropped by CiteAb from the following publication (<https://journal.frontiersin.org/article/10.3389/fnmol.2017.00229/full>) licensed under a CC-BY license.



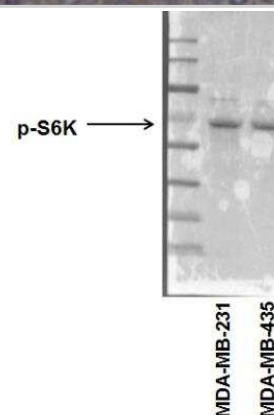
Immunocytochemistry/Immunofluorescence: p70 S6 Kinase/S6K [p Thr389/412] Antibody [NB600-1049] - Analysis of Phospho-p70 S6 Kinase in 293 cells.



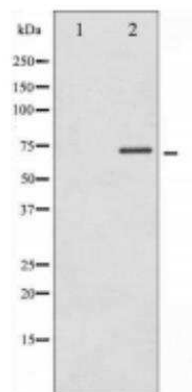
Immunohistochemistry: p70 S6 Kinase/S6K [p Thr389/412] Antibody [NB600-1049] - Human liver cancer tissue sections



Western Blot: p70 S6 Kinase/S6K [p Thr389/412] Antibody [NB600-1049] - Analysis of phospho-p70 S6 Kinase in human breast cancer cell lines (MDA-MB-231 and MDA-MB-435) using p70 S6 Kinase/S6K [p Thr390, p Thr412] Antibody. Image from verified customer review.



Western Blot: p70 S6 Kinase/S6K [p Thr389/412] Antibody [NB600-1049] - Analysis of p70 S6 Kinase phosphorylation expression in Insulin treated Jurkat whole cell lysates. The lane on the left is treated with the antigen-specific peptide.



Publications

V Sharma, M Makhdoomi, L Singh, P Kumar, N Khan, S Singh, HN Verma, K Luthra, S Sarkar, D Kumar Trehalose limits opportunistic mycobacterial survival during HIV co-infection by reversing HIV-mediated autophagy block Autophagy, 2020-02-20;0(0):1-20. 2020-02-20 [PMID: 32079455]

Hashikawa-Hobara N, Otsuka A, Okujima C, Hashikawa N CGRP overexpression does not alter depression-like behavior in mice PeerJ 2021-07-02 [PMID: 34249519] (WB)

Chen S, Lu XT, He TT et al. Betaine Delayed Muscle Loss by Attenuating Samtor Complex Inhibition for mTORC1 Signaling Via Increasing SAM Level Molecular nutrition & food research 2021-06-01 [PMID: 34061446]

Gao K, Niu J, Dang X Wnt-3a improves functional recovery through autophagy activation via inhibiting the mTOR signaling pathway after spinal cord injury Neurosci. Lett. 2020-08-17 [PMID: 32818590] (WB, Rat)

Zhang Q, Wei D, Tan M et al. Transgenic expression of Sag/Rbx2 E3 causes early stage tumor promotion, late stage cytogenesis and acinar loss in the Kras-PDAC model Neoplasia 2020-04-24 [PMID: 32339950] (IHC-P, IF/IHC, Mouse)

Lee HJ, Jung YH, Choi GE et al. O-cyclic phytosphingosine-1-phosphate stimulates HIF1 alpha-dependent glycolytic reprogramming to enhance the therapeutic potential of mesenchymal stem cells Cell Death Dis 2019-08-05 [PMID: 31383843] (WB)

Lee KH, Lee S, Lee HJ et al. Amyloid B1-42 (AB1-42) Induces the CDK2-Mediated Phosphorylation of Tau through the Activation of the mTORC1 Signaling Pathway While Promoting Neuronal Cell Death Front. Mol. Neurosci. 2017-07-24 [PMID: 28790888] (WB, Human)



Procedures

Western Blot Protocol for S6K Antibody (NB600-1049)

Western Blot Protocol for S6K Antibody (NB600-1049):

Western Blot Protocol

Solutions and Reagents:

Transfer Buffer:

25 mM Tris-base (pH 8.5), 0.2 M Glycin, 20% methanol

Cell Extract Buffer:

50 mM Pipes/NaOH (pH 6.5), 2 mM EDTA, 0.1% Chaps, 5 mM DTT, 20 ug/ml Leupeptin, 10 ug/ml Pepstatin, 10 ug/ml aprotinin, and 1 mM PMSF.

SDS-PAGE Loading Buffer:

62.5 mM Tris-HCl, (pH 6.8), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue

10X TBS (Tris-Buffered Saline):

To prepare 1 liter of 10X TBS: 24.2 g Tris-base, 80 g NaCl, adjust pH to 7.6 with HCl (use at 1X).

TBS/T Washing Buffer:

1X TBS, 0.1% Tween-20

Blocking Buffer:

1X TBS/T with 5% BSA

Primary Antibody Dilution Buffer:

1X TBS/T with 5% BSA

Western Blot Detection:

Protein marker, secondary anti-rabbit antibody conjugated to HRP, chemiluminescent reagent, peroxide.

Protein Blotting:

1. Treat cells by adding fresh media containing regulator for desired time.
 2. Aspirate media from cultures, wash cells with 1X PBS, aspirate. Scrape cells into PBS and spin down to pellet.
 3. Lyse cells by adding Cell Extract Buffer (one volume of cell pellet, or 100 ul per well of 6-well plate or 500 ul per plate of 10 cm² plate). Freeze and thaw 3 times. Centrifuge lysate at microcentrifuge using top speed. (~14000 rpm). Keep the supernatant and discard the pelleted cell debris.
 4. Add SDS Loading Buffer and heat to 95-100°C for 5 minutes, cool on ice.
 5. Microcentrifuge for 5 minutes.
 6. Load 5-20 ul onto SDS-PAGE gel (10 cm x 10 cm).
- Note: We recommend loading prestained molecular weight markers to verify electrotransfer.
7. Electrotransfer to nitrocellulose membrane.

Membrane Blocking & Antibody Incubations:

Note: Volumes are for 10 cm x 10 cm of membrane. For different sized membranes, adjust volumes accordingly

1. Incubate membrane in 25 ml of Blocking Buffer for 1 hour at room temperature.
2. Wash 3 times for 5 min each with 15 ml of TBS/T.
3. Incubate membrane and NB600-1049 1-2 ug/ml in 10 ml Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
4. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
5. Incubate membrane with HRP-conjugated secondary antibody in 10 ml of Blocking Buffer with gentle agitation for 1 hour at room temperature.
6. Wash membrane as in step 4.
7. Proceed with detection.

Detection of Proteins:

1. Remove the wash buffer and place the blot in a plastic bag or clean tray containing chemiluminescent working solution (0.125 ml/cm²) and peroxide (ECL detection method).
2. Rotate the bag or tray to allow the solution to cover the surface of the membrane for 1-5 minutes.
3. Remove blot from bag or tray and place it between two pieces of write-on acetate transparency film. Smooth over covered blot to remove air bubbles and excess substrate.
Expose to X-ray film. An initial exposure of 10-60 seconds is recommended for film.



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Limitations

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Primary Antibodies are guaranteed for 1 year from date of receipt.

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