

Datasheet for 600-401-B30S**p90 RSK1 phospho S732 Antibody****Overview**

Description:	Anti-p90 RSK1 pS732 (RABBIT) Antibody - 600-401-B30S
Item No.:	600-401-B30S
Size:	25 µL
Applications:	ELISA, WB, IP
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Ribosomal S6 Kinase 1 (RSK1, S6K-alpha 1, 90 kDa Ribosomal Protein S6 Kinase 1, MAP kinase-activated protein kinase 1a, or MAPKAPK1A) is an immediate downstream effector of mitogen activated protein kinases and therefore promotes cell proliferation and survival. It has serine/threonine kinase activity and may play a role in mediating the growth-factor and stress-induced activation of the transcription factor CREB. The C-terminal region of RSK1 is reported to be an ERK docking site, where serine 732 phosphorylation status is critical for RSK1 activation. When serine 732 is not phosphorylated, ERK1/2 binds to the ERK docking site of RSK1, and upon stimulation, activates RSK1. The activated RSK1 then autophosphorylates serine 732, leading to the dissociation of ERK from RSK1 and termination of activation by ERK. RSK1 is studied in cancer research and is known to inactive tumor suppressor complexes and death kinases.
Synonyms:	rabbit anti-p90 RSK1 pS732 antibody, rabbit anti-RSK1 pS732 antibody, Ribosomal S6 Kinase 1, RSK-1, S6K-alpha 1, 90 kDa Ribosomal Protein S6 Kinase 1, MAP kinase-activated protein kinase 1a, MAPK-activated protein kinase 1a, p90-RSK 1, p90S6K, MAPKAP kinase 1a, MAPKAPK-1a, Ribosomal S6 kinase 1, RSK 1, RPS6KA1, MAPKAPK1A
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	RPS6KA1
Reactivity:	Human

PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic phospho-peptide corresponding to a region near the C-terminal end of human RSK1 protein.
Purity/Specificity:	This product was affinity purified from monospecific antiserum by immunoaffinity chromatography using phosphorylated peptide coupled to agarose beads followed by solid phase adsorption against non phosphorylated peptide. This antibody is specific for human p90 RSK1 protein phosphorylated at S732. A BLAST analysis was used to suggest cross reactivity with p90 RSK1 from human, rat, mouse, horse, bovine, and dog based on 100% homology with the immunizing sequence. Partially cross reactivity may occur against opossum and chicken based on >90% homology with the immunizing sequence. Cross reactivity with p90 RSK1 pS732 from other sources has not been determined.
Relevant Links:	• UniProtKB - Q15418 • NCBI - NP_001006666.1 • GeneID - 6195

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IP (Based on references)
Application Note:	This affinity-purified antibody has been tested for use in ELISA and western blotting. Specific conditions for reactivity should be optimized by the end user. By western blot a band approximately 90 kDa in size corresponding to p90 RSK1 pS732 protein is expected in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5000-1:50,000
WB:	1:1000-1:3000

Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.51 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide

Stabilizer: None

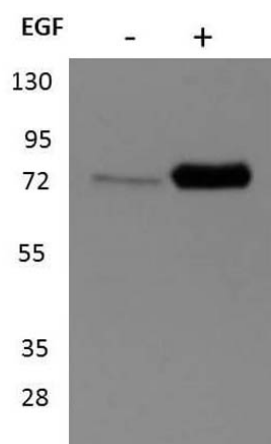
Shipping & Handling

Shipping Condition: Dry Ice

Storage Condition: Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.

Expiration: Expiration date is three (3) months from date of receipt.

Images



Western Blot

Western Blot of Rabbit anti-p90 RSK1 antibody.

Lane 1: unstimulated HEK293T cell lysates.

Lane 2: EGF stimulated HEK293T cell lysates.

Load: 35 µg per lane.

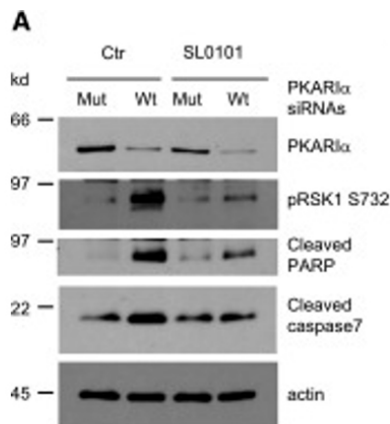
Primary antibody: p90 RSK1 antibody at 1:1000 for overnight at 4°C.

Secondary antibody: peroxidase conjugated secondary antibody and ECL.

Block: 5% BLOTTO overnight at 4°C.

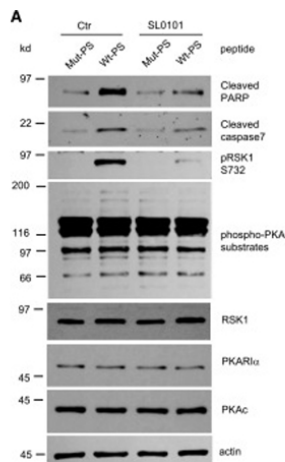
Predicted/Observed size: 90 kDa, ~90 kDa for p90 RSK1.

Other band(s): none.



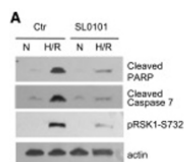
Western Blot

Silencing of PKAR1α in HL-1 cells induces RSK1-mediated apoptosis. (A) 48 hours after transfection with mutant (Mut) or Wt siRNA against PKAR1α, HL-1 cells were serum starved and incubated with or without SL0101 (50 μM) for 24 hours. Cell lysates were subjected to Western analysis. Fig 2. PMID: 24307699



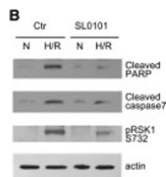
Western Blot

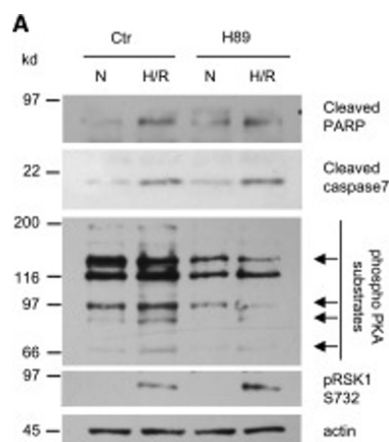
A cell-permeable peptide (Wt-PS) that disrupts interactions between PKAR1α and RSK1 activates RSK1 and induces apoptosis in HL-1 cardiomyocytes. (A) Serum-starved HL-1 cells were incubated with 2 μM of the peptide corresponding to the pseudosubstrate region of PKAR1α (Wt-PS) or the same peptide-harboring mutations (Mut-PS) either in the presence or absence of SL0101 (50 μM) for 24 hours. Cell lysates were subjected to Western analysis for the various indicated proteins as well as phospho-S732 RSK1 and phospho-PKA substrates Fig 3. PMID: 24307699



Western Blot

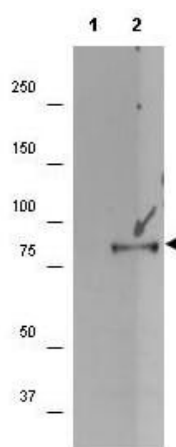
H/R-induced apoptosis is attenuated by RSK1 inhibitor SL0101. ARVMs (A) or HL-1 cells (B) were treated with or without SL0101 (50 μM) and exposed to normoxia or hypoxia (1% O₂) for 24 hours, followed by reoxygenation for 1 hour. Cell lysates were subjected to Western analysis. Fig 4. PMID: 24307699





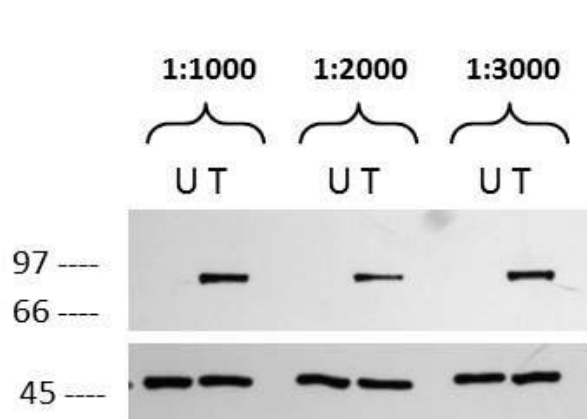
Western Blot

Elevated PKA activity does not contribute toward the H/R-induced apoptosis in cardiomyocytes. (A) After serum starvation, HL-1 cells were treated with or without H89 (10 μ M) and exposed to normoxia or hypoxia (1% O₂) for 24 hours, followed by reoxygenation for 1 hour. Cell lysates were subjected to Western analysis for the indicated proteins. Arrows depict the changes in intensity of the PKA substrate bands. Fig 6. PMID: 24307699



Western Blot

Western blot using Rockland's affinity purified anti-p90 RSK1 pS732 antibody shows detection of a band ~90 kDa in size corresponding to phosphorylated p90 RSK1 (arrowhead) in EGF stimulated (lane 2) HEK293T cell lysates prepared from cells grown in the absence of serum for 12 h. No staining is observed in similarly prepared lysates derived from unstimulated (control) cells (lane 1). After transfer, the membrane was blocked overnight followed by reaction with the primary antibody at a 1:1,000 dilution. Detection occurred using a peroxidase conjugated secondary antibody and ECL. Personal Communication. Kuldeep Patel, Loyola University Medical Center, Maywood, IL.



Western Blot

Western blot using Rockland's affinity purified anti-p90 RSK1 pS732 antibody. Lane 1-2: HEK293T (U) untreated or (T) treated with EGF. Lane 3-4: HEK293T (U) untreated or (T) treated with EGF. Lane 5-6: HEK293T (U) untreated or (T) treated with EGF. Load: 15 μ g per lane. Actin used as a loading control. Blocking: 5% milk. Primary Antibody: Anti-RSK1-pS732 1:1000, 1:2000, or 1:3000 O/N. Secondary Antibody: Goat Anti-Rabbit IgG 1:5000 for 2 hours. Predicted Size: ~90 kDa in size corresponding to phosphorylated p90 RSK1 in EGF stimulated. Personal Communication. Kuldeep Patel, Loyola University Medical Center, Maywood, IL.

References

- Gao X, Lin B, Sadayappan S, Patel TB. Interactions between the regulatory subunit of type I protein kinase A and p90 ribosomal S6 kinase1 regulate cardiomyocyte apoptosis. *Mol Pharmacol.* (2014)

Disclaimer

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