

# Anti-Phospho-RPS6 (S240 + S244) Antibody [PS00-65]

## HA721589



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IHC-P, IF-Cell
<b>Molecular Wt:</b>	Predicted band size: 29 kDa.
<b>Clone number:</b>	PS00-65

**Description:** Ribosomes, the organelles that catalyze protein synthesis, consist of a small 40S subunit and a large 60S subunit. Together these subunits are composed of 4 RNA species and approximately 80 structurally distinct proteins. This gene encodes a cytoplasmic ribosomal protein that is a component of the 40S subunit. The protein belongs to the S6E family of ribosomal proteins. It is the major substrate of protein kinases in the ribosome, with subsets of five C-terminal serine residues phosphorylated by different protein kinases. Phosphorylation is induced by a wide range of stimuli, including growth factors, tumor-promoting agents, and mitogens. Dephosphorylation occurs at growth arrest. The protein may contribute to the control of cell growth and proliferation through the selective translation of particular classes of mRNA. As is typical for genes encoding ribosomal proteins, there are multiple processed pseudogenes of this gene dispersed through the genome.

**Immunogen:** Synthetic peptide within Human RPS6 aa 200 to the C-terminus (phospho S240 + S244).

**Positive control:** A431 cell lysates, human colon carcinoma tissue, human pancreas tissue, mouse hippocampus tissue, mouse brain tissue, SK-Br-3.

**Subcellular location:** Cytosol, Endoplasmic reticulum, Nucleus.

**Database links:** SwissProt P62753 Human | P62754 Mouse | P62755 Rat

**Recommended Dilutions:**

<b>WB</b>	1:1,000
<b>IHC-P</b>	1:5,000-10,000
<b>IF-Cell</b>	1:50

**Storage Buffer:** PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% SodiumAzide.

**Storage Instruction:** Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

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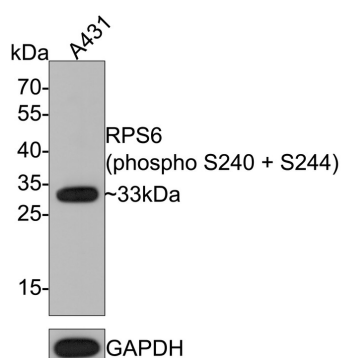
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## Images



**Fig1:** Western blot analysis of Phospho-RPS6 (S240 + S244) on A431 cell lysates with Rabbit anti-Phospho-RPS6 (S240 + S244) antibody (HA721589) at 1/1,000 dilution.

Lysates/proteins at 10 µg/Lane.

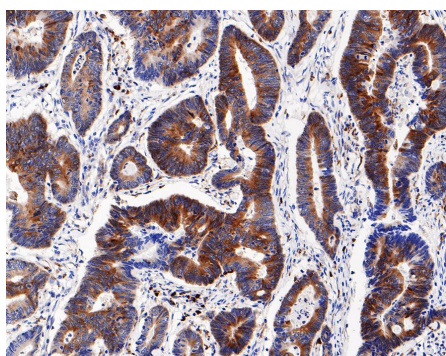
Predicted band size: 29 kDa

Observed band size: 33 kDa

Exposure time: 2 minutes;

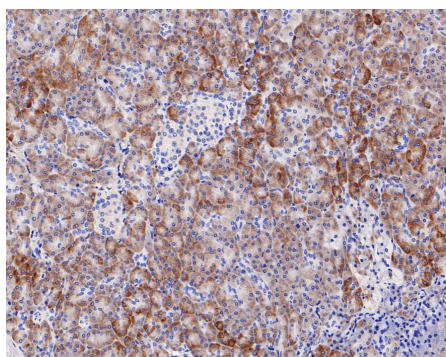
12% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDm/TBST for 1 hour at room temperature. The primary antibody (HA721589) at 1/1,000 dilution was used in 5% NFDm/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Phospho-RPS6 (S240 + S244) antibody (HA721589) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721589) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig3:** Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-Phospho-RPS6 (S240 + S244) antibody (HA721589) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721589) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

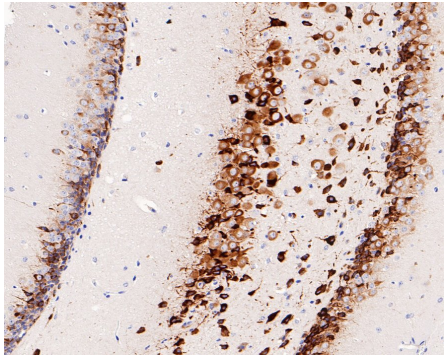
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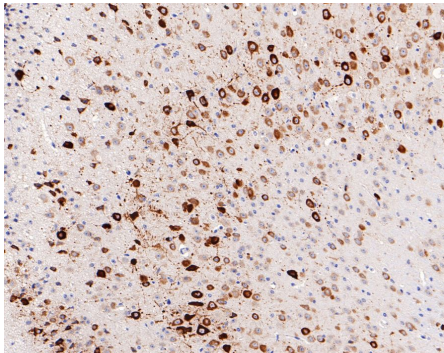
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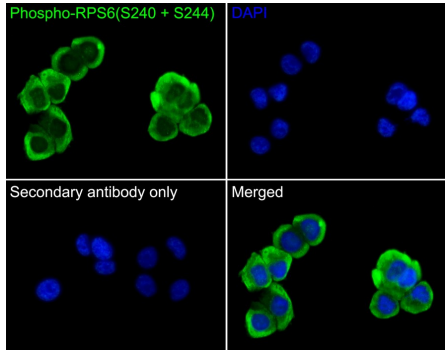
**Fig4:** Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-Phospho-RPS6 (S240 + S244) antibody (HA721589) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721589) at 1/10,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig5:** Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Phospho-RPS6 (S240 + S244) antibody (HA721589) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721589) at 1/10,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig6:** Immunocytochemistry analysis of SK-Br-3 cells labeling Phospho-RPS6 (S240 + S244) with Rabbit anti-Phospho-RPS6 (S240 + S244) antibody (HA721589) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-RPS6 (S240 + S244) antibody (HA721589) at 1/50 dilution in 2% negative goat serum overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

**Note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

## Background References

1. Yan M. et al. mTORC1/rpS6 signaling complex modifies BTB transport function: an in vivo study using the adjuvin model. *Am J Physiol Endocrinol Metab.* 2019 Jul
2. Wu S. et al. mTORC1/rpS6 and spermatogenic function in the testis-insights from the adjuvin model. *Reprod Toxicol.* 2019 Oct

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