# Anti-RPS6 Antibody [A6B9]

## HA600084



Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 29 kDa
Clone number:	A6B9
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.
lmmunogen:	Recombinant protein within human RPS6 aa 1-150.
Positive control:	HeLa cell lysate, MCF7 cell lysate, HepG2 cell lysate, HEK-293 cell lysate, HCT 116 cell lysate, THP-1 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, human brain tissue lysate, human kidney tissue lysate, HeLa, human breast carcinoma tissue, human pancreas tissue, mouse colon tissue, rat brain tissue, rat hippocampus tissue.
Subcellular location:	Cytosol, Endoplasmic reticulum, Nucleus.
Database links:	SwissProt: P62753 Human   P62754 Mouse   P62755 Rat
Recommended Dilutions: WB IF-Cell IHC-P FC	1:500-1:2,000 1:200 1:2,000 1:1,000
Storage Buffer:	1*TBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!\!{\rm C}$ after thawing. Aliquot store at -20 $^\circ\!\!{\rm C}$ . Avoid repeated freeze / thaw cycles.
Purity:	Protein G affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn



#### Images



Fig1: Western blot analysis of RPS6 on different lysates with Mouse anti-RPS6 antibody (HA600084) at 1/1,000 dilution.

Lane 1: HeLa cell Iysate (20 µg/Lane) Lane 2: MCF7 cell Iysate (20 µg/Lane) Lane 3: HepG2 cell Iysate (20 µg/Lane) Lane 4: HEK-293 cell Iysate (20 µg/Lane) Lane 5: HCT 116 cell Iysate (20 µg/Lane) Lane 6: THP-1 cell Iysate (20 µg/Lane) Lane 7: NIH/3T3 cell Iysate (20 µg/Lane) Lane 8: PC-12 cell Iysate (20 µg/Lane) Lane 9: Human brain tissue Iysate (40 µg/Lane) Lane 10: Human kidney tissue Iysate (40 µg/Lane)

Predicted band size: 29 kDa Observed band size: 33 kDa

Exposure time: 3 minutes;

4-20% 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 (HA600084) at 1/1,000 dilution was used in 5% NFDM/TBST at  $4^{\circ}$ C overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunocytochemistry analysis of HeLa cells labeling RPS6 with Mouse anti-RPS6 antibody (HA600084) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 1% BSA for 30 minutes at room temperature. Cells were then incubated with Mouse anti-RPS6 antibody (HA600084) at 1/200 dilution in 1% BSA overnight at 4  $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor M 488, HA1125) 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.

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**Fig3:** Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Mouse anti-RPS6 antibody (HA600084) at 1/2,000 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 (HA600084) at 1/2,000 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.



**Fig4:** Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Mouse anti-RPS6 antibody (HA600084) at 1/2,000 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 (HA600084) at 1/2,000 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 colon tissue with Mouse anti-RPS6 antibody (HA600084) at 1/2,000 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 (HA600084) at 1/2,000 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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**Fig6:** Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-RPS6 antibody (HA600084) at 1/2,000 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 (HA600084) at 1/2,000 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.



**Fig7:** Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Mouse anti-RPS6 antibody (HA600084) at 1/2,000 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 (HA600084) at 1/2,000 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.



Fig8: Flow cytometric analysis of HeLa cells labeling RPS6.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA600084, 1µg/mL) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor <sup>TM</sup> 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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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 adjudin model. Am J Physiol Endocrinol Metab. 2019 Jul
- Wu S. et. al. mTORC1/rpS6 and spermatogenic function in the testis-insights from the adjudin model. Reprod Toxicol. 2019 Oct

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

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