Anti-p70 S6 Kinase Antibody [PSH13-50]

HA723539

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 59 kDa
Clone number:	PSH13-50

Description: Ribosomal protein S6 kinase beta-1 (S6K1), also known as p70S6 kinase (p70S6K, p70-S6K), is an enzyme (specifically, a protein kinase) that in humans is encoded by the RPS6KB1 gene. It is a serine/threonine kinase that acts downstream of PIP3 and phosphoinositide-dependent kinase-1 in the PI3 kinase pathway. As the name suggests, its target substrate is the S6 ribosomal protein. Phosphorylation of S6 induces protein synthesis at the ribosome. The phosphorylation of p70S6K at threonine 389 has been used as a hallmark of activation by mTOR and correlated with autophagy inhibition in various situations. However, several recent studies suggest that the activity of p70S6K plays a more positive role in the increase of autophagy. This gene encodes a member of the S6K family of serine/threonine kinases, which phosphorylate several residues of the S6 ribosomal protein. The kinase activity of this protein leads to an increase in protein synthesis and cell proliferation. Amplification of the region of DNA encoding this gene and overexpression of this kinase are seen in some breast cancer cell lines. Alternate translational start sites have been described and alternate transcriptional splice variants have been observed but have not been thoroughly characterized.

Immunogen: Recombinant protein within Human p70 S6 Kinase aa 1-75 and aa 454-525.

Positive control: HeLa cell lysate, MCF7 cell lysate, 293T cell lysate, HL-60 cell lysate, Neuro-2a cell lysate, PC-12 cell lysate, COS-1 cell lysate, HeLa, Neuro-2a, C6.

Subcellular location: Synapse, synaptosome, Mitochondrion outer membrane, Mitochondrion; Nucleus, Cytoplasm.

Database links: SwissProt: P23443 Human | Q8BSK8 Mouse | P67999 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100-1:250
FC	1:1,000
IP	1-2μg/sample

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

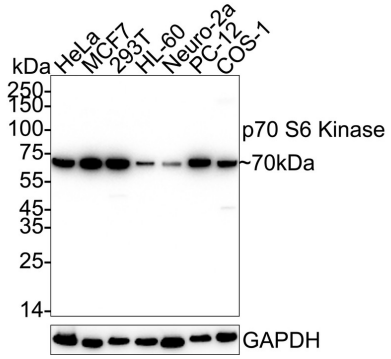

Fig1: Western blot analysis of p70 S6 Kinase on different lysates with Rabbit anti-p70 S6 Kinase antibody (HA723539) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: MCF7 cell lysate
Lane 3: 293T cell lysate
Lane 4: HL-60 cell lysate
Lane 5: Neuro-2a cell lysate
Lane 6: PC-12 cell lysate
Lane 7: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 70 kDa

Exposure time: 25 seconds; ECL: K1801;

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 (HA723539) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

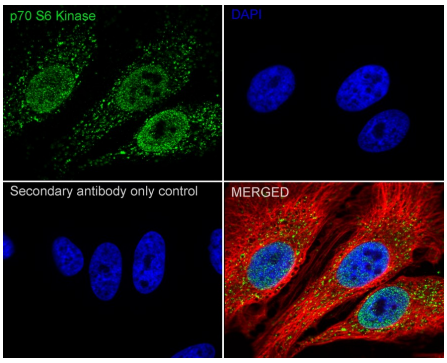

Fig2: Immunocytochemistry analysis of HeLa cells labeling p70 S6 Kinase with Rabbit anti-p70 S6 Kinase antibody (HA723539) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-p70 S6 Kinase antibody (HA723539) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. 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.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

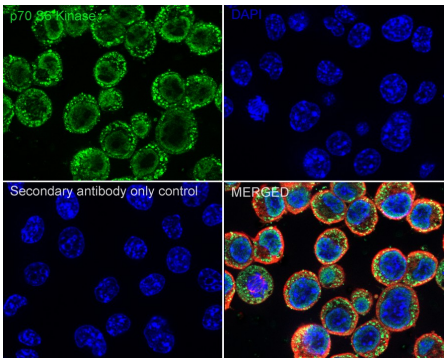

Fig3: Immunocytochemistry analysis of Neuro-2a cells labeling p70 S6 Kinase with Rabbit anti-p70 S6 Kinase antibody (HA723539) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-p70 S6 Kinase antibody (HA723539) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

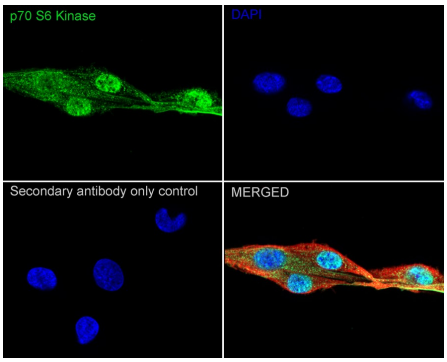

Fig4: Immunocytochemistry analysis of C6 cells labeling p70 S6 Kinase with Rabbit anti-p70 S6 Kinase antibody (HA723539) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-p70 S6 Kinase antibody (HA723539) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

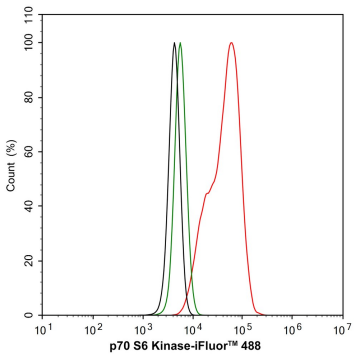

Fig5: Flow cytometric analysis of Neuro-2a cells labeling p70 S6 Kinase.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723539, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

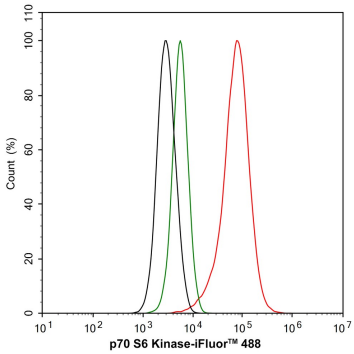

Fig6: Flow cytometric analysis of C6 cells labeling p70 S6 Kinase.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723539, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

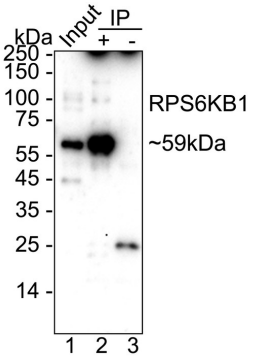

Fig7: p70 S6 Kinase was immunoprecipitated from 0.2 mg HeLa cell lysate with HA723539 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723539 at 1/5,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA723539 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA723539 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 minutes; ECL: K1802

Background References

1. Artemenko M et al. p70 S6 kinase as a therapeutic target in cancers: More than just an mTOR effector. Cancer Lett. 2022 Jun
2. Fan Y et al. Phosphoproteomic Analysis of Neonatal Regenerative Myocardium Revealed Important Roles of Checkpoint Kinase 1 via Activating Mammalian Target of Rapamycin C1/Ribosomal Protein S6 Kinase b-1 Pathway. Circulation. 2020 May