Anti-Phospho-p70 S6 Kinase (T389) Antibody [PSH02-23]

HA721803

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 59 kDa
Clone number:	PSH02-23

Description: This gene encodes a member of the ribosomal S6 kinase family of serine/threonine kinases. The encoded protein responds to mTOR (mammalian target of rapamycin) signaling to promote protein synthesis, cell growth, and cell proliferation. Activity of this gene has been associated with human cancer. Alternatively spliced transcript variants have been observed. The use of alternative translation start sites results in isoforms with longer or shorter N-termini which may differ in their subcellular localizations. There are two pseudogenes for this gene on chromosome 17.

Immunogen: Synthetic peptide within human P70 S6 Kinase aa 371-420 / 525.

Positive control: MCF7 cell lysate, MCF7 treated with 20% FBS overnight then add 100nM Calyculin A for 30 minutes cell lysate, NIH/3T3 treated with 20% FBS overnight then add 100nM Calyculin A for 30 minutes cell lysate, human breast carcinoma tissue, MCF7 starved for 4 hours then treated with 200ng/mL EGF for 15 minutes cell lysate, C6 cell lysate, C6 treated with 100nM Calyculin A for 30 minutes cell lysate, human liver carcinoma tissue, 293T cell lysate, 293T serum starved overnight cell lysate, 293T serum starved overnight then treated with 100nM Insulin for 15 minutes cell lysate, MCF7 cells treated with 20% FBS overnight then add 100nM Calyculin A for 30 minutes.

Subcellular location: Synapse, synaptosome, Mitochondrion outer membrane, Mitochondrion.

Database links: SwissProt: P23443 Human | Q8BSK8 Mouse
Entrez Gene: 83840 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:100
IHC-P	1:1,000

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

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

Purity: Protein A affinity purified.

Images

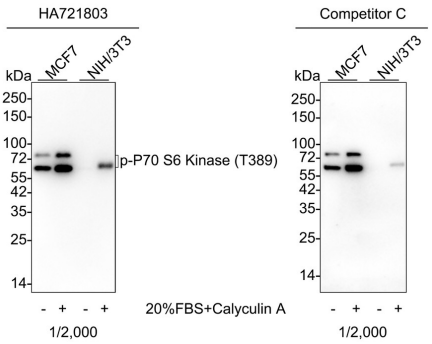

Fig1: Western blot analysis of Phospho-p70 S6 Kinase (T389) on different lysates with Rabbit anti-Phospho-p70 S6 Kinase (T389) antibody (HA721803) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: MCF7 treated with 20% FBS overnight then add 100nM Calyculin A for 30 minutes cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 20% FBS overnight then add 100nM Calyculin A for 30 minutes cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 70/85 kDa

Exposure time: Lane 1-4 (left): 1 minute 2 seconds; Lane 1-4 (right): 3 minutes; 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 (HA721803) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were 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.

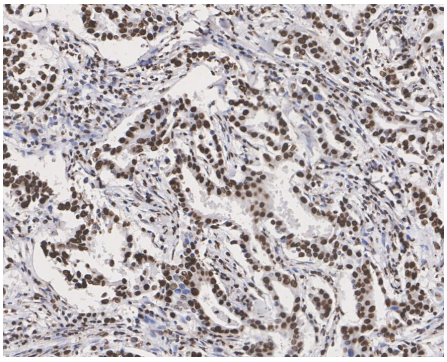

Fig2: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Phospho-p70 S6 Kinase (T389) antibody (HA721803) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721803) at 1/1,000 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.

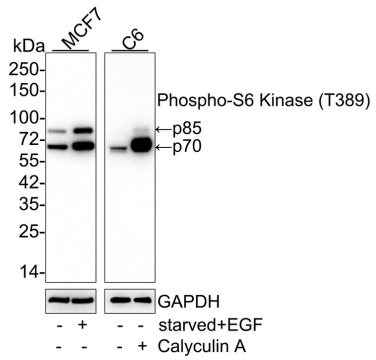

Fig3: Western blot analysis of Phospho-p70 S6 Kinase (T389) on different lysates with Rabbit anti-Phospho-p70 S6 Kinase (T389) antibody (HA721803) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: MCF7 starved for 4 hours then treated with 200ng/mL EGF for 15 minutes cell lysate
Lane 3: C6 cell lysate
Lane 4: C6 treated with 100nM Calyculin A for 30 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 70/85 kDa

Exposure time: 46 seconds; ECL: K1802;

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 (HA721803) at 1/1,000 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.

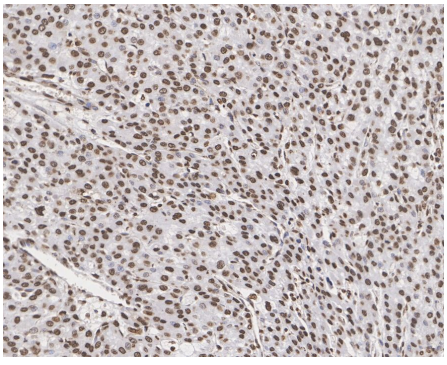

Fig4: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue with Rabbit anti-Phospho-p70 S6 Kinase (T389) antibody (HA721803) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721803) at 1/1,000 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.

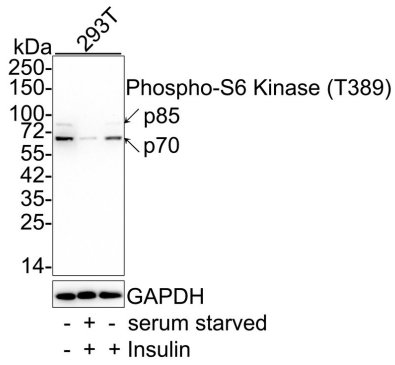

Fig5: Western blot analysis of Phospho-p70 S6 Kinase (T389) on different lysates with Rabbit anti-Phospho-p70 S6 Kinase (T389) antibody (HA721803) at 1/2,000 dilution.

Lane 1: 293T cell lysate
Lane 2: 293T serum starved overnight cell lysate
Lane 3: 293T serum starved overnight then treated with 100nM Insulin for 15 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 70/85 kDa

Exposure time: 3 minutes 20 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 (HA721803) 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.

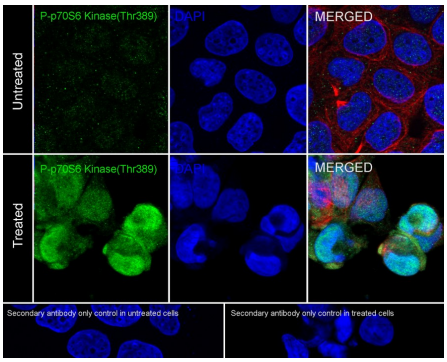

Fig6: Immunocytochemistry analysis of MCF7 cells treated with or without 20% FBS overnight then add 100nM Calyculin A for 30 minutes labeling Phospho-p70 S6 Kinase (T389) with Rabbit anti-Phospho-p70 S6 Kinase (T389) antibody (HA721803) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Phospho-p70 S6 Kinase (T389) antibody (HA721803) 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 (M1305-2, 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.

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. Wu J et al. CircRNA Uxs1/miR-335-5p/PGF axis regulates choroidal neovascularization via the mTOR/p70 S6k pathway. Transl Res. 2023 Jun