

S6 Polyclonal Antibody

Catalog Number PA5-29760

Product data sheet

Details		Species Reactivity	
Size	100 µL	Species reactivity	Human, Mouse, Zebrafish
Host/Isotope	Rabbit / IgG	Tested Applications	
Class	Polyclonal	Immunohistochemistry - Free Floating (IHC (Free))	Dilution * Assay-dependent
Type	Antibody	Immunohistochemistry (Paraffin) (IHC (P))	1:100-1:1,000
Immunogen	Recombinant protein fragment corresponding to a region within amino acids 1 and 146 of Human RPS6	Western Blot (WB)	1:500-1:3,000
		Immunocytochemistry (ICC/IF)	1:100-1:1,000
Conjugate	Unconjugated	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Form	Liquid		
Concentration	2.34 mg/mL		
Purification	Antigen affinity chromatography		
Storage buffer	PBS		
Contains	no preservative		
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.		

Product specific information

Recommended positive controls: HeLa, HepG2, Mouse brain. Predicted reactivity: Mouse (100%), Rat (100%), Zebrafish (96%), Drosophila (84%), Xenopus laevis (96%), Dog (100%), Chicken (98%), Bovine (100%). Store product as a concentrated solution. Centrifuge briefly prior to opening the vial.

Background/Target Information

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.

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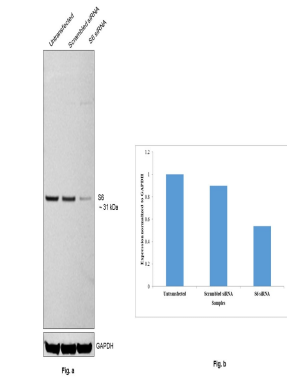
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S6 Antibody (PA5-29760) in WB

Western blot was performed using Anti-S6 Polyclonal Antibody(Product # PA5-29760) and a 31kDa band corresponding to S6 was observed across all cell lines tested. Whole cell extracts (30 µg lysate) of A-431 (Lane 1), HeLa (Lane 2), Hep G2 (Lane 3), Jurkat (Lane 4), NTERA-2 cl.D1 (Lane 5) and U-87 MG (Lane 6) were electrophoresed using NuPAGE™ 10% Bis-Tris Protein Gel (Product # NP0302BOX). Resolved proteins were then transferred onto a Nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:500 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).

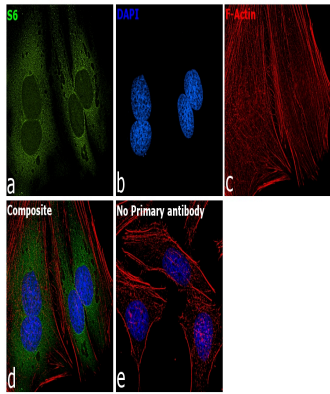
S6 Antibody (PA5-29760)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with S6 siRNA and decrease in signal intensity was observed in Western Blot application using Anti-S6 Polyclonal Antibody (Product # PA5-29760). {KD}



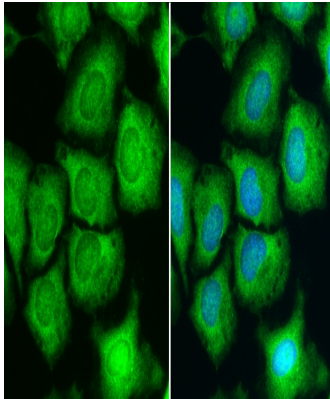
S6 Antibody (PA5-29760) in ICC/IF

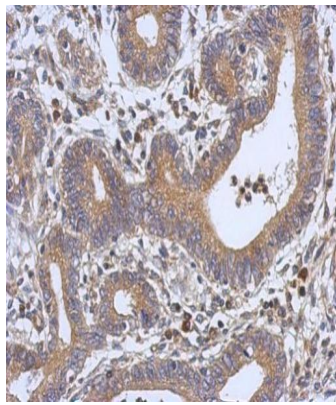
Immunofluorescence analysis of S6 was performed using 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with S6 Polyclonal Antibody (Product # PA5-29760) at 1:100 dilution in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32790), (1:2000 dilution), for 45 minutes at room temperature (Panel a: Green). Nuclei (Panel b:Blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: Red) was stained with Rhodamine Phalloidin (Product # R415, 1:300 dilution). Panel d represents the merged image showing cytosolic localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



S6 Antibody (PA5-29760) in ICC/IF

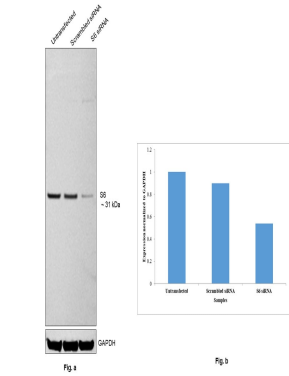
Immunocytochemistry-Immunofluorescence analysis of S6 was performed in HeLa cells fixed in 4% paraformaldehyde at RT for 15 min. Green: S6 Polyclonal Antibody (Product # PA5-29760) diluted at 1:500. Blue: Hoechst 33342 staining.





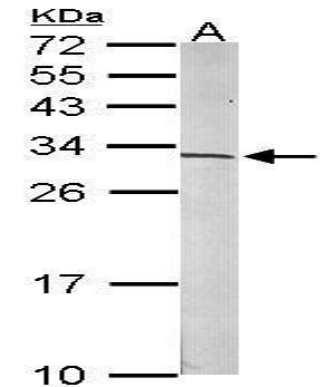
S6 Antibody (PA5-29760) in IHC (P)

Immunohistochemical analysis of paraffin-embedded human colon carcinoma, using RPS6 (Product # PA5-29760) antibody at 1:500 dilution. Antigen Retrieval: EDTA based buffer, pH 8.0, 15 min.



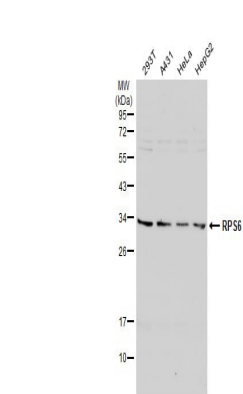
S6 Antibody (PA5-29760) in WB

Knockdown of S6 was achieved by transfecting HeLa with S6 specific siRNAs (Silencer® select Product # S12270, S12272). Western blot analysis (Fig. a) was performed using whole cell extracts from the S6 knockdown cells (lane 3), non-targeting scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with S6 Polyclonal Antibody (Product # PA5-29760, 1:500 dilution) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to S6.



S6 Antibody (PA5-29760) in WB

Western Blot using S6 Polyclonal Antibody (Product # PA5-29760). Sample (50 µg of whole cell lysate). Lane A: mouse brain. 12% SDS PAGE. S6 Polyclonal Antibody (Product # PA5-29760) diluted at 1:500. The HRP-conjugated anti-rabbit IgG antibody was used to detect the primary antibody.



S6 Antibody (PA5-29760) in WB

Western Blot using S6 Polyclonal Antibody (Product # PA5-29760). Various whole cell extracts (30 µg) were separated by 12% SDS-PAGE, and the membrane was blotted with S6 Polyclonal Antibody (Product # PA5-29760) diluted at 1:500. The HRP-conjugated anti-rabbit IgG antibody was used to detect the primary antibody.

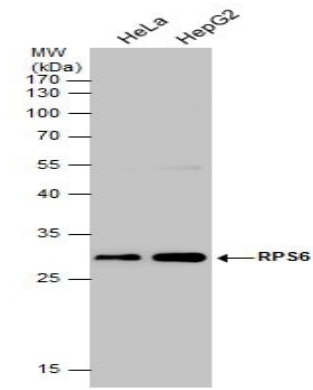
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