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# S6 Recombinant Rabbit Monoclonal Antibody (9H8L2)

**Catalog Number** 701374

**Product data sheet**

| Details | |
|---|---|
| **Size** | 100 µg |
| **Host/Isotope** | Rabbit / IgG |
| **Class** | Recombinant Monoclonal |
| **Type** | Antibody |
| **Clone** | 9H8L2 |
| **Immunogen** | Recombinant protein corresponding to amino acids 2-249 of human RPS6. |
| **Conjugate** | Unconjugated |
| **Form** | Liquid |
| **Concentration** | 0.5 mg/mL |
| **Purification** | Protein A |
| **Storage buffer** | PBS |
| **Contains** | 0.09% sodium azide |
| **Storage Conditions** | Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles. |

| Species Reactivity | |
|---|---|
| **Species reactivity** | Dog, Human |

| Tested Applications | Dilution * |
|---|---|
| **ChIP assay (ChIP)** | 1 µL |
| **Flow Cytometry (Flow)** | 1-3 µg/1x10^6 cells |
| **Western Blot (WB)** | 0.5-1 µg/mL |
| **Immunocytochemistry (ICC/IF)** | 1 µg/mL |

* 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.

## Product specific information

Intact IgG appears on a non-reducing gel as ~150 kDa band and upon reduction generating a ~25 kDa light chain band and a ~50 kDa heavy chain. Recombinant rabbit monoclonal antibodies are produced using in vitro expression systems. The expression systems are developed by cloning in the specific antibody DNA sequences from immunoreactive rabbits. Then, individual clones are screened to select the best candidates for production. The advantages of using recombinant rabbit monoclonal antibodies include: better specificity and sensitivity, lot-to-lot consistency, animal origin-free formulations, and broader immunoreactivity to diverse targets due to larger rabbit immune repertoire.

## 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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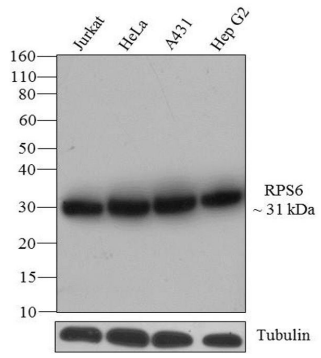
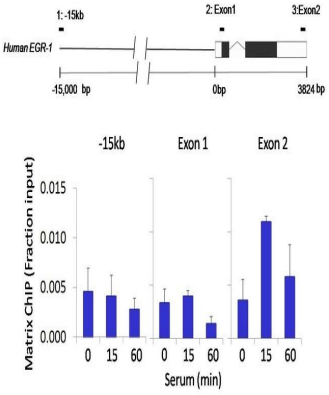
## Product Images For S6 Recombinant Rabbit Monoclonal Antibody (9H8L2)



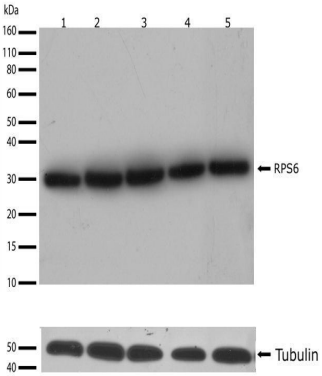

### S6 Antibody (701374) in WB

Western blot analysis of RPS6 was performed by loading 20 µg of Jurkat (lane1), HeLa (lane2), A431 (lane3) and Hep G2 (lane4) cell lysates using Novex® NuPAGE® 4-12 % Bis-Tris gel (Product # NP0321BOX), XCell SureLock™ Electrophoresis System (Product # EI0002), Novex® Sharp Pre-Stained Protein Standard (LC5800), and iBlot® Dry Blotting System (IB21001). Proteins were transferred to a nitrocellulose membrane and blocked with 5 % skim milk for 1 hour at room temperature. RPS6 was detected at ~31 kDa using RPS6 Rabbit Monoclonal Antibody (Product # 701374) at 0.5-1 µg/mL in 2.5 % skim milk at 4°C overnight on a rocking platform. Goat Anti-Rabbit IgG-HRP Secondary Antibody (G21234) at 1:5000 dilution was used and chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



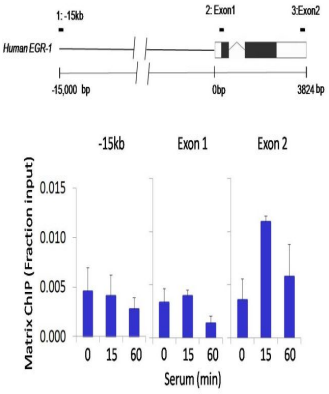

### S6 Antibody (701374)

Antibody specificity was demonstrated through detection of enrichment of the target protein at specific gene loci. Chromatin Immunoprecipitation (ChIP) was performed using Recombinant Rabbit Monoclonal Anti-S6 Antibody (Product # 701374) with relevant positive and negative target genes/ binding sites. {RE}




### S6 Antibody (701374) in WB

Western blot analysis of RPS6 in whole cell extracts from Jurkat, HeLa treated withTNFa, untreated HeLa, A431, and HepG2 cells (lanes 1-5 respectively) using a RPS6 recombinant rabbit monoclonal antibody (Product # 701374) at a dilution of 1 µg/mL. Tubulin was used as a loading control and detected with an anti-tubulin antibody. Samples were detected using chemiluminescence (ECL). Results show a band at ~31kDa.




### S6 Antibody (701374) in ChIP

Chromatin immunoprecipitation analysis of S6 Ribosomal Protein performed using cross-linked chromatin from 1 x 10^6 HCT116 human colon carcinoma cells treated with serum for 0, 15, and 60 minutes. Immunoprecipitation was performed using a multiplex microplate Matrix ChIP assay (see reference for Matrix ChIP protocol: http://www.ncbi.nlm.nih.gov/pubmed/22098709) with 1.0 µL/100 µL well volume of a S6 Ribosomal Protein rabbit monoclonal antibody (Product # 701374). Chromatin aliquots from ~1 x 10^5 cells were used per ChIP pull-down. Quantitative PCR data were done in quadruplicate using 1 µL of eluted DNA in 2 µL SYBR real-time PCR reactions containing primers to amplify -15kb upstream of the human Egr-1 locus, or exon-1 or exon-2 of Egr-1. PCR calibration curves were generated for each primer pair from a dilution series of sheared total genomic DNA. Quantitation of immunoprecipitated chromatin is presented as signal relative to the total amount of input chromatin. Results represent the mean +/- SEM for three experiments. A schematic representation of the Egr-1 locus is shown above the data where boxes represent exons (black boxes = translated regions, white boxes = untranslated regions), the zigzag line represents an intron, and the straight line represents upstream sequence. Regions amplified by Egr-1 primers are represented by black bars. Data courtesy of the Innovators Program.

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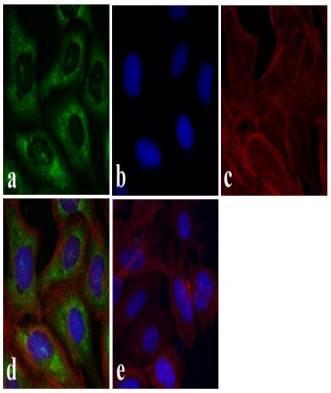
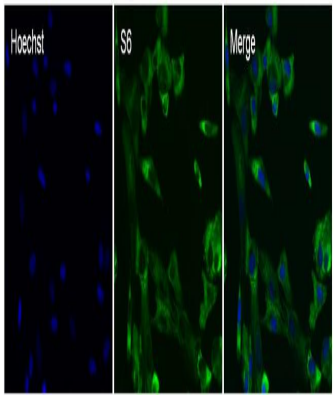


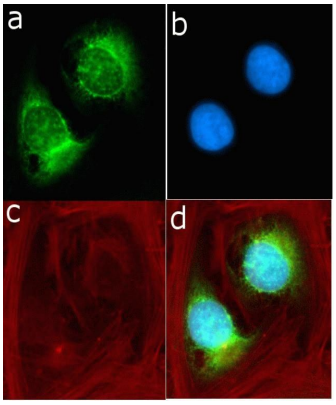

**S6 Antibody (701374) in ICC/IF**

Immunofluorescent analysis of RPS6 was done on 70% confluent log phase U-2 OS cells. The cells were fixed with 4% paraformaldehyde for 15 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with RPS6 Recombinant Rabbit Monoclonal Antibody (Product # 701374) at 1 µg/mL in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor 488 Goat anti-Rabbit IgG Secondary Antibody (Product # A-11008) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor 594 Phalloidin (Product # A12381). Panel d is a merged image showing cytoplasmic localization and panel e is a no primary antibody control. The images were captured at 20X magnification.



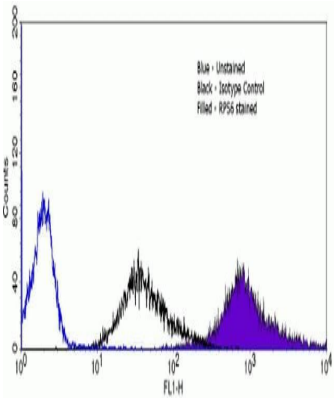

**S6 Antibody (701374) in ICC/IF**

Immunofluorescent analysis of ribosomal protein 6 (green) in MDCK cells. The cells were permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and blocked with 3% BSA in PBS (Product # 37525) for 15 minutes at room temperature. Cells were stained with a S6 Rabbit monoclonal antibody (Product # 701374), at a concentration of 10 µg /mL in blocking buffer for at least 1 hour at room temperature, and then incubated with a Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034) at a dilution of 1:1000 for 30 minutes at room temperature (green). Nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ToxInsight Instrument at 20X magnification.




**S6 Antibody (701374) in ICC/IF**

Immunofluorescent analysis of RPS6 in U2OS cells using a RPS6 recombinant rabbit monoclonal antibody (Product # 701374) followed by detection using an Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (green) (Image A). Nuclei were stained using DAPI (Image B) and actin stained with Alexa Fluor 594 phalloidin (red) (image C). Image D is a composite image showing cytoplasmic localization of RPS6.




**S6 Antibody (701374) in Flow**

Flow cytometry analysis of RPS6 in Hela cells using a RPS6 recombinant rabbit monoclonal antibody (Product # 701374). Cells were fixed and permeabilized using FIX & PERM (Product # GAS-004) reagent, and detection was performed using an Alexa Fluor 488 goat anti-rabbit IgG (right peak) compared to an isotype control (middle peak, black) and a control without primary antibody (left peak, blue).

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