

Phospho-S6 Ribosomal Protein (Ser235/236) (91B2) Rabbit mAb

- Small 100 µl (10 western blots)
- Large 300 µl (30 western blots)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

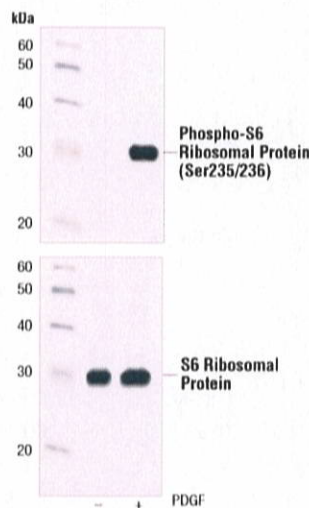
Entrez-Gene ID #6194
Swiss-Prot Acc. #P62753

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IHC-F, IF-IC Endogenous	H, M, R	32 kDa	Rabbit IgG**

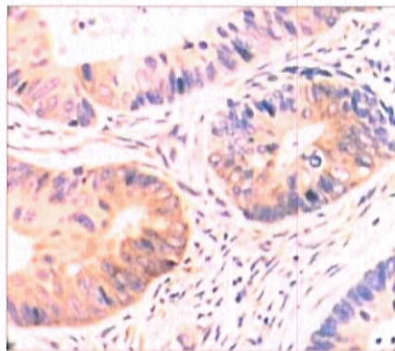
Background: One way that growth factors and mitogens effectively promote sustained cell growth and proliferation is by upregulating mRNA translation (1,2). Growth factors and mitogens induce the activation of p70 S6 kinase and the subsequent phosphorylation of the S6 ribosomal protein. Phosphorylation of S6 ribosomal protein correlates with an increase in translation of mRNA transcripts that contain an oligopyrimidine tract in their 5' untranslated regions (2). These particular mRNA transcripts (5TOP) encode proteins involved in cell cycle progression as well as ribosomal proteins and elongation factors necessary for translation (2,3). Important S6 ribosomal protein phosphorylation sites include several residues (Ser235, Ser236, Ser240 and Ser244) located within a small, carboxy-terminal region of the S6 protein (4,5).

Specificity/Sensitivity: Phospho-S6 Ribosomal Protein (Ser235/236) (91B2) Rabbit mAb detects endogenous levels of ribosomal protein S6 only when phosphorylated at serines 235 and 236.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser235 and Ser236 of human ribosomal protein S6.



Western blot analysis of extracts from NIH/3T3 cells, untreated or PDGF-treated (100ng/ml, 20 min), using Phospho-S6 Ribosomal protein (Ser235/236) (91B2) Rabbit mAb (upper) or S6 Ribosomal Protein (5G10) Rabbit mAb #2217 (lower).



Immunohistochemical analysis of paraffin-embedded human colon carcinoma, showing cytoplasmic localization, using Phospho-S6 Ribosomal Protein (Ser235/236) (91B2) Rabbit mAb.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunohistochemistry (Paraffin)	1:75
IHC protocol: Unmasking buffer/Antibody diluent Citrate/ SignalStain™ Antibody Diluent #8112	
Immunohistochemistry (Frozen)	1:75
Fixative: 10% Neutral buffered formalin	
Immunofluorescence (IF-IC)	1:100

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

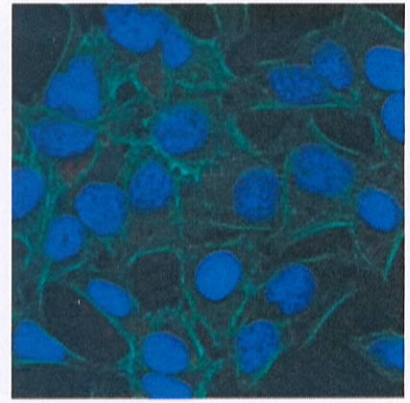
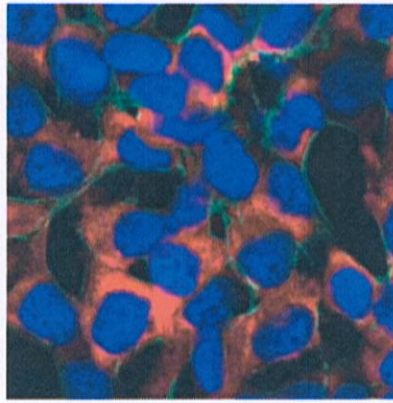
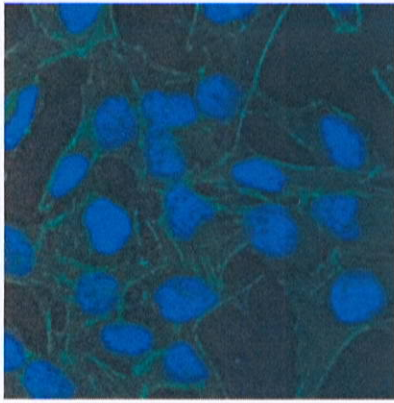
Background References:

- Dufner, A. and Thomas, G. (1999) *Exp. Cell Res.* 253, 100-109.
- Peterson, R.T. and Schrelber, S.L. (1998) *Curr. Biol.* 8, R248-R250.
- Jefferies, H. B. et al. (1997) *EMBO J.* 16, 3693-3704.
- Ferrari, S. et al. (1991) *J. Biol. Chem.* 266, 22770-22775.
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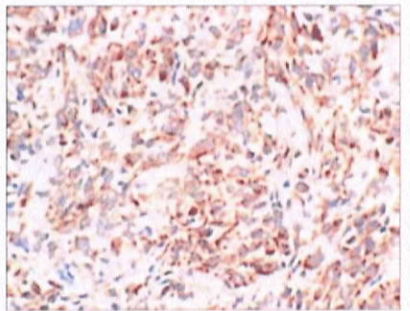
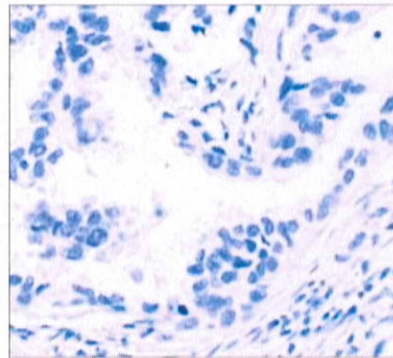
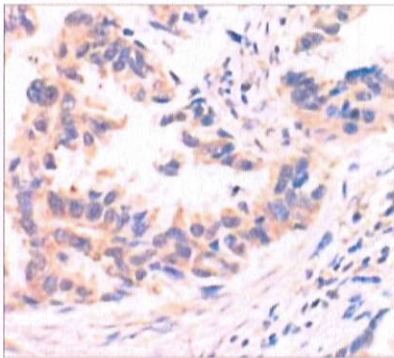
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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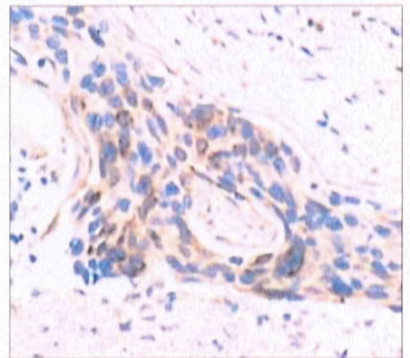
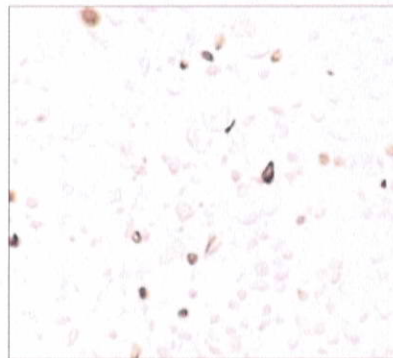
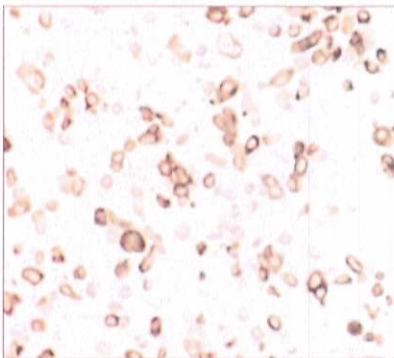
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey MI—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Confocal immunofluorescent images of HeLa cells serum-starved for 20 hrs (left), 20% serum-treated (center), or 20% serum-treated after preincubation with Rapamycin (FHAP/mTOR Inhibitor) #9904 and labeled with Phospho-S6 Ribosomal Protein (Ser235/236) (91B2) Rabbit mAb (red). Actin filaments have been labeled with fluorescein phalloidin. Blue pseudocolor = DRAQ5[®] #4084 (fluorescent DNA dye).



Immunohistochemical analysis of frozen U-87MG xenograft, showing cytoplasmic localization using Phospho-S6 Ribosomal Protein (Ser235/236) (91B2) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded LNCaP cells, untreated (left) or rapamycin-treated (right), using Phospho-S6 Ribosomal Protein (Ser235/236) (91B2) Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using Phospho-S6 Ribosomal Protein (Ser235/236) (91B2) Rabbit mAb.