

**#2211** Store at -20°C

# Phospho-S6 Ribosomal Protein (Ser235/236) Antibody

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

rev. 04/06/11



**Orders** ■ 877-616-CELL (2355)  
 orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
 info@cellsignal.com  
**Web** ■ www.cellsignal.com

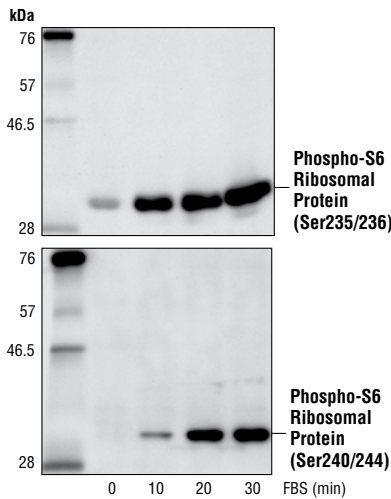
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IHC-P, IHC-F, IF-IC, F Endogenous	H, M, R, Mk, Sc, (C, X)	32 kDa	Rabbit**

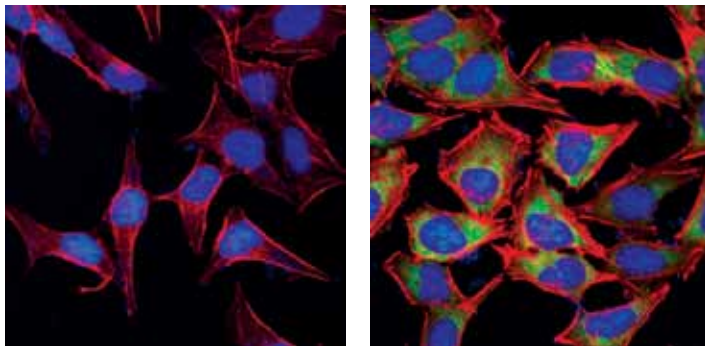
**Background:** One way that growth factors and mitogens effectively promote sustained cell growth and proliferation is by upregulating mRNA translation (1,2). Growth factors and mitogens induce the activation of p70 S6 kinase and the subsequent phosphorylation of the S6 ribosomal protein. Phosphorylation 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 (5'TOP) 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) Antibody detects endogenous levels of ribosomal protein S6 only when phosphorylated at serine 235 and 236. This antibody does not detect ribosomal protein S6 phosphorylated at other sites.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser235 and Ser236 of human ribosomal protein S6. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from 293 cells, untreated or treated with 20% FBS for the indicated time, using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody (upper) or Phospho-S6 Ribosomal Protein (Ser240/244) Antibody #2215 (lower).



Confocal immunofluorescent analysis of HeLa cells, serum-starved (left) or 20% serum-treated (right), using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

**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.

**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.

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

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. 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
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:50
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Immunohistochemistry (Frozen)	1:100
Fixative:	10% Neutral buffered formalin
Immunofluorescence (IF-IC)	1:100
Flow Cytometry	1:50

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

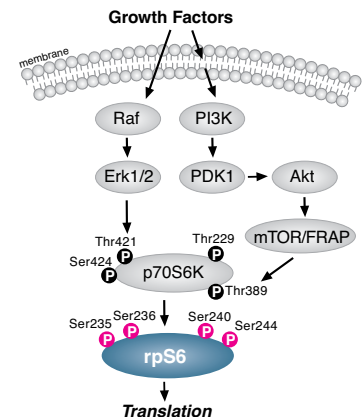
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

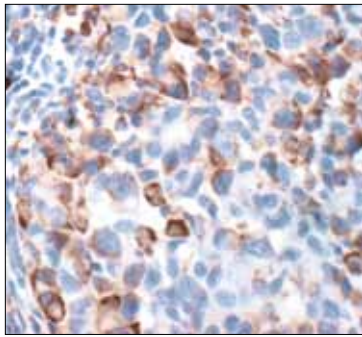
**Background References:**

- Dufner, A. and Thomas, G. (1999) *Exp. Cell Res.* 253, 100–109.
- Peterson, R.T. and Schreiber, 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.
- Flotow, H. and Thomas, G. (1992) *J. Biol. Chem.* 267, 3074–3078.

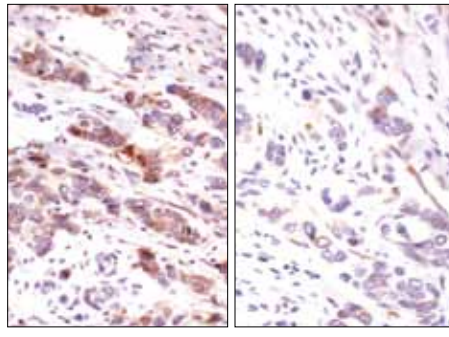
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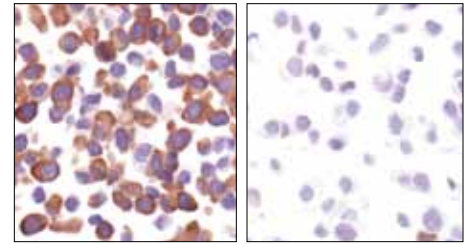




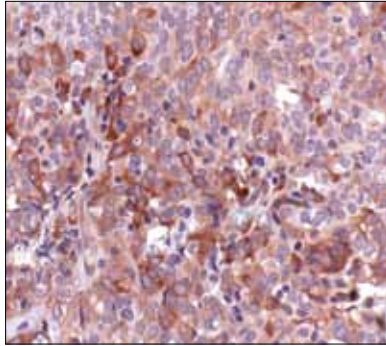
Immunohistochemical staining of paraffin-embedded human melanoma, showing cytoplasmic localization of phosphorylated S6 ribosomal protein, using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody.



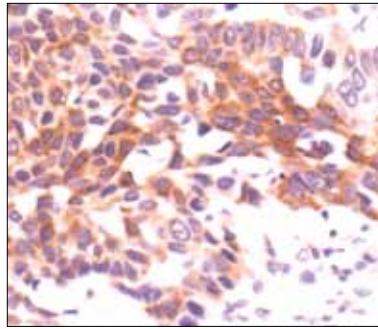
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, untreated (left) or calf-intestinal phosphatase (CIP) treated (right), using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody.



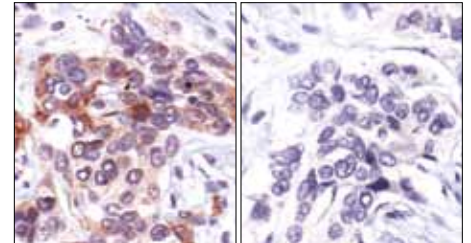
Immunohistochemical analysis of paraffin-embedded LNCaP cells, untreated (left) or rapamycin-treated (right), using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody.



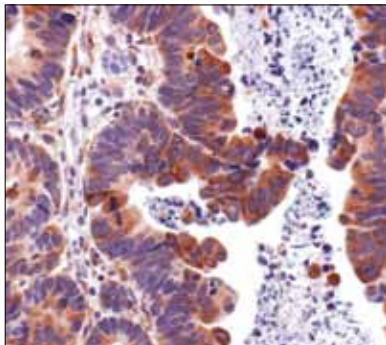
Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma, using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody.



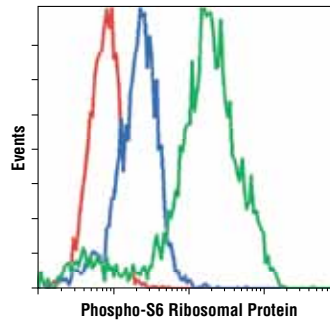
Immunohistochemical analysis of paraffin-embedded human lung carcinoma, using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody.



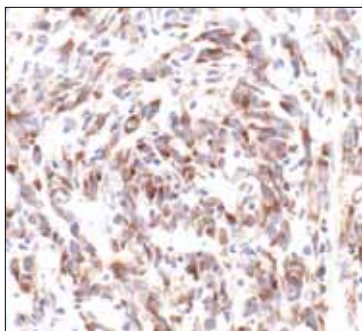
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody preincubated with control peptide (left) or Phospho-S6 Ribosomal Protein (Ser235/236) Blocking Peptide #1220 (right).



Immunohistochemical analysis of paraffin-embedded human renal cell carcinoma, using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody.



Flow cytometric analysis of NIH/3T3 cells, U0126- and Rapamycin-treated (blue) or serum-treated (green), using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody compared to a nonspecific negative control antibody (red).



Immunohistochemical analysis of frozen U87MG xenograft, using Phospho-S6 Ribosomal Protein (Ser235/236) Antibody.