

**#4858** Store at **-20°C**

# Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb

- Small 100 µl (20 western blots)
- Large 300 µl (60 western blots)



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

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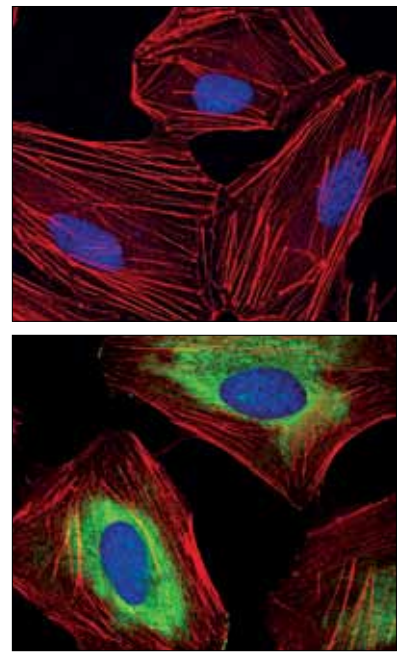
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

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

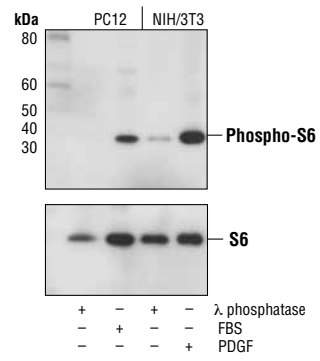
**Background:** One way that growth factors and mitogens effectively promote sustained cell growth and proliferation is by upregulating mRNA translation (1,2). Growth factors and mitogens induce the activation of p70 S6 kinase and the subsequent phosphorylation of the S6 ribosomal protein. Phosphorylation 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 (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) (D57.2.2E) XP® Rabbit mAb detects endogenous levels of ribosomal protein S6 only when phosphorylated at Ser235 and 236.

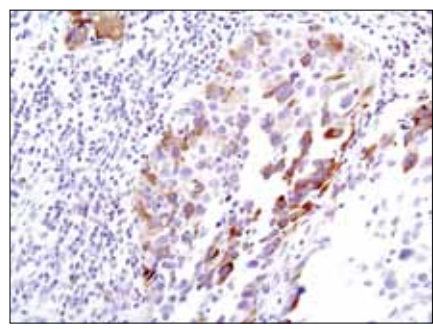
**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser235 and Ser236 of human ribosomal protein S6.



Confocal immunofluorescent analysis of HeLa cells, rapamycin-treated (upper) or 20% serum-treated (lower), using Phospho-S6 Ribosomal protein (Ser235/Ser236) (D57.1.5) XP® Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Western blot analysis of extracts from PC12 and NIH/3T3 cells, treated with λ phosphatase, FBS or PDGF as indicated, using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb (upper) or S6 Ribosomal Protein (5G10) Rabbit mAb #2217 (lower).



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb.

**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	1:2000
Immunohistochemistry (Paraffin)	1:400†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunohistochemistry (Frozen)	1:400
Fixative:	10% NBF
Immunofluorescence (IF-IC)	1:100
Flow Cytometry	1:25

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

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

**Background References:**

- (1) Dufner, A. and Thomas, G. (1999) *Exp. Cell Res.* 253, 100–109.
- (2) Peterson, R.T. and Schreiber, S.L. (1998) *Curr. Biol.* 8, R248–R250.
- (3) Jefferies, H.B. et al. (1997) *EMBO J.* 16, 3693–3704.
- (4) Ferrari, S. et al. (1991) *J. Biol. Chem.* 266, 22770–22775.
- (5) Flotow, H. and Thomas, G. (1992) *J. Biol. Chem.* 267, 3074–3078.

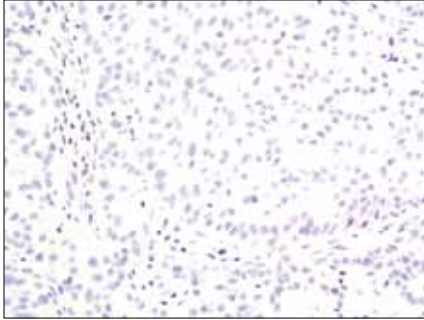
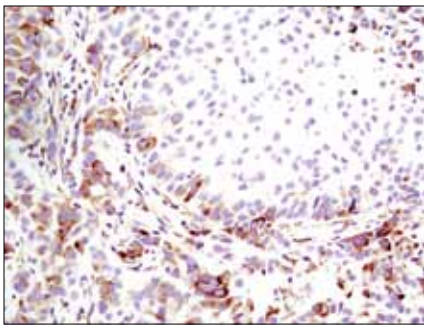
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

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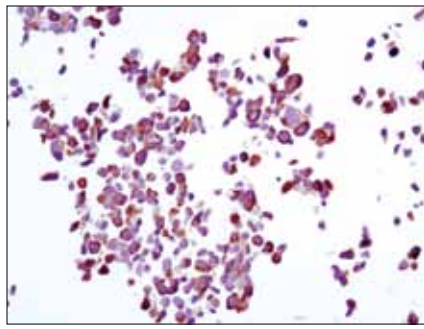
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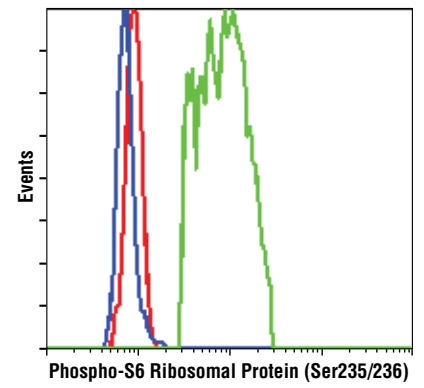
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish 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.



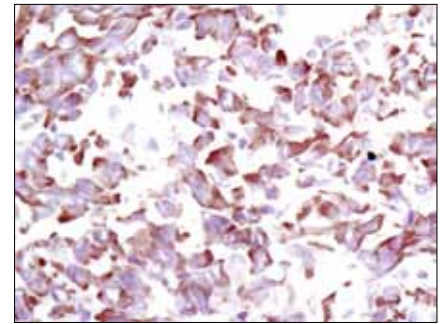
Immunohistochemical analysis of paraffin-embedded A549 xenograft, untreated (upper) or  $\lambda$  phosphatase-treated (lower), using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP<sup>®</sup> Rabbit mAb.



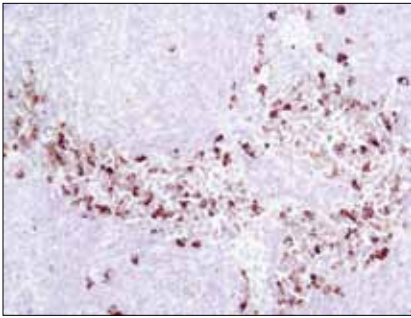
Immunohistochemical analysis of paraffin-embedded LNCaP cells, untreated (upper) or rapamycin-treated (lower), using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP<sup>®</sup> Rabbit mAb.



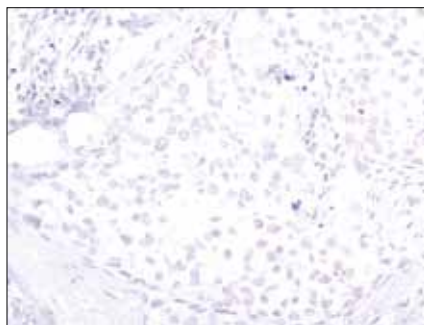
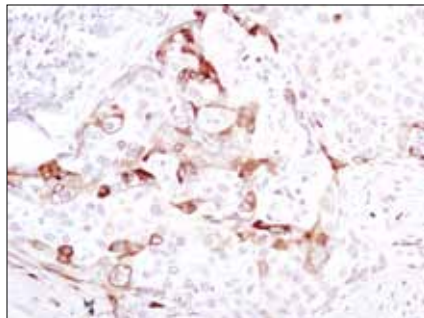
Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002, wortmannin and U0126 (blue), using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP<sup>®</sup> Rabbit mAb compared to a nonspecific negative control antibody (red).



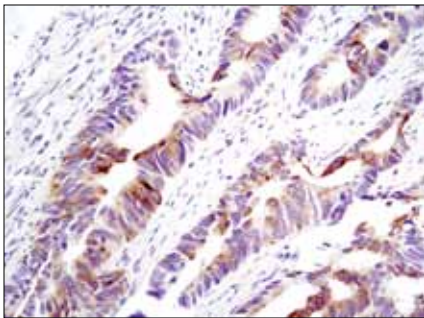
Immunohistochemical analysis of frozen U-87MG xenograft using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP<sup>®</sup> Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded mouse spleen using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP<sup>®</sup> Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP<sup>®</sup> Rabbit mAb in the presence of control peptide (upper) or Phospho-S6 Ribosomal Protein (Ser235/236) Blocking Peptide #1220 (lower).



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP<sup>®</sup> Rabbit mAb.