

#5364 Store at -20°C

# Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP® Rabbit mAb



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**For Research Use Only. Not For Use In Diagnostic Procedures.**

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

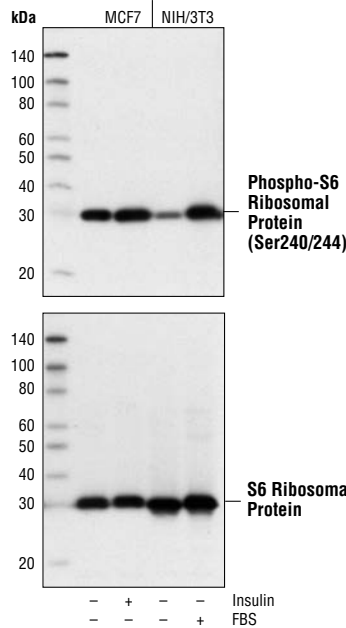
**Background:** One way that growth factors and mitogens effectively promote sustained cell growth and proliferation is by upregulating mRNA translation (1,2). Growth factors and mitogens induce the activation of p70 S6 kinase and the subsequent phosphorylation of the S6 ribosomal protein. Phosphorylation 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 (Ser240/244) (D68F8) XP® Rabbit mAb detects endogenous levels of ribosomal protein S6 only when phosphorylated at Ser240 and Ser244.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser240 and Ser244 of human ribosomal protein S6.

**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, v22770-22775.
- Flotow, H. and Thomas, G. (1992) *J. Biol. Chem.* 267, 3074-3078.



Western blot analysis of extracts from MCF7 and NIH/3T3 cells, treated with 100 nM insulin (10 min) or 20% FBS (30 min) as indicated, using Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP® Rabbit mAb (upper) or S6 Ribosomal Protein (5G10) Rabbit mAb #2217 (lower).

Confocal immunofluorescent analysis of HeLa cells, insulin-treated (upper) and LY294002-treated (#9901, lower), using Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP® Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Entrez-Gene ID #6194  
UniProt ID #P62753

**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.

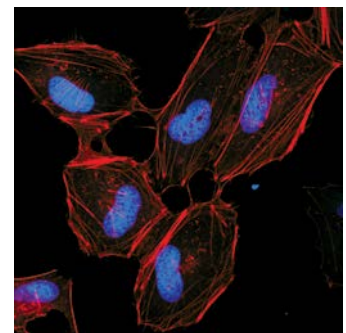
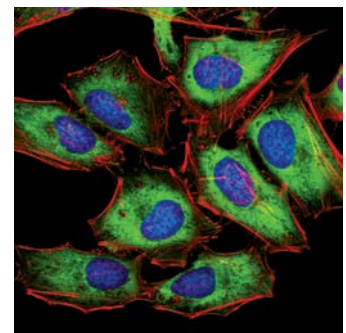
\*Species cross-reactivity is determined by western blot.

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

**Recommended Antibody Dilutions:**

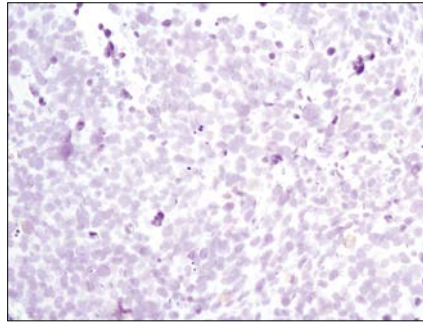
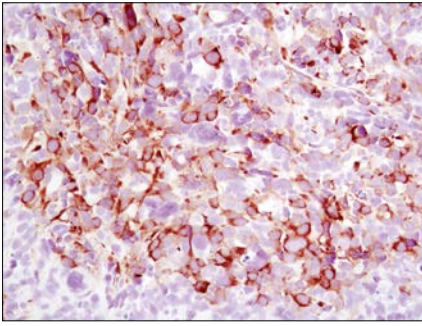
Western blotting	1:1000
Immunohistochemistry (Paraffin)	1:1000
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunofluorescence (IF-IC)	1:800
Flow Cytometry	1:200

For product specific protocols and a complete listing of recommended companion products please see the product web page at [www.cellsignal.com](http://www.cellsignal.com).

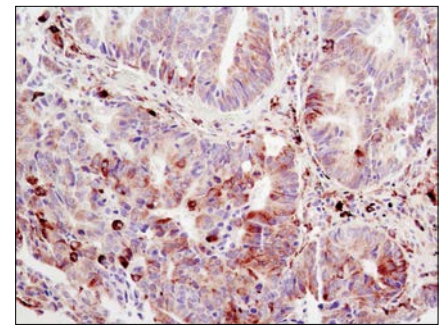


**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.**

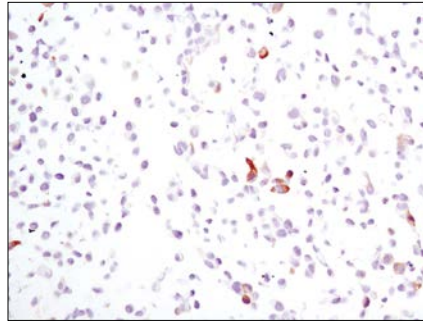
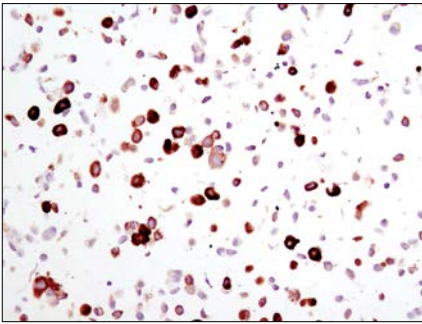
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish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



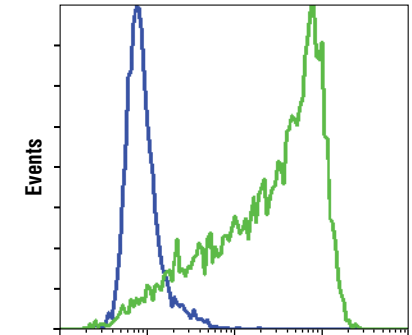
Immunohistochemical analysis of paraffin-embedded Rh30 xenograft, control (left) or rapamycin-treated (right), using Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP<sup>®</sup> Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP<sup>®</sup> Rabbit mAb.

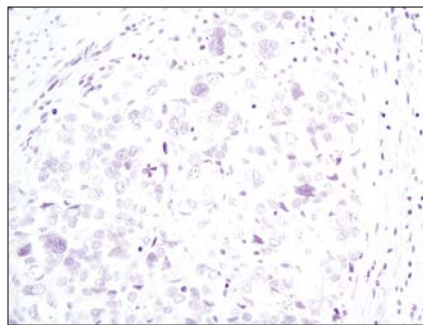
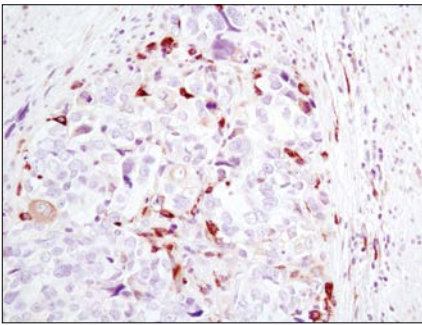


Immunohistochemical analysis on SignalSlide<sup>®</sup> Phospho-Akt (Ser473) IHC Controls #8101 (paraffin-embedded LNCaP cell pellets +/- LY294002) using Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP<sup>®</sup> Rabbit mAb.

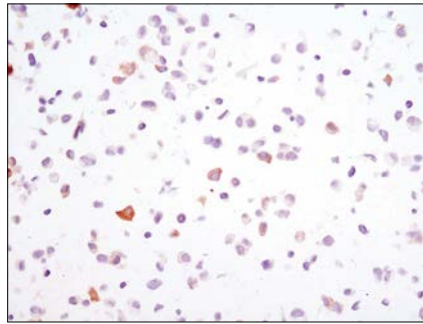
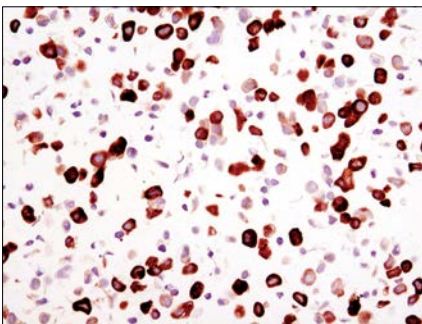


**Phospho-S6 Ribosomal Protein (Ser240/244)**

Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002, wortmannin and U0126 (blue), using Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP<sup>®</sup> Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP<sup>®</sup> Rabbit mAb in the presence of control peptide (left) or antigen-specific peptide (right).



Immunohistochemical analysis of paraffin-embedded LNCaP cell pellets, control (left) or rapamycin-treated (right), using Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP<sup>®</sup> Rabbit mAb.