

#4851 Store at 4°C

Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate)

✓ 100 µl (50 tests)
New more concentrated formulation

rev. 05/17/10



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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.

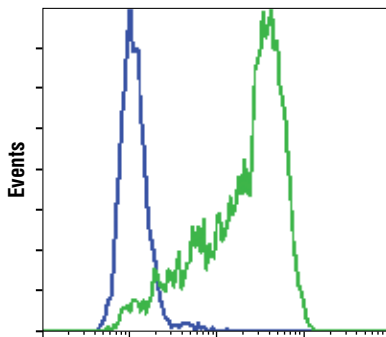
Applications	Species Cross-Reactivity*	Isotype
IF-IC, IF-P, F Endogenous	H, M, R, Mk, Sc	Rabbit IgG

Description: This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb #4858 reacts with human, monkey, mouse, rat and *S. cerevisiae* phospho-S6 Ribosomal protein. CST expects that Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate) will also recognize phospho-S6 in these species.

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).

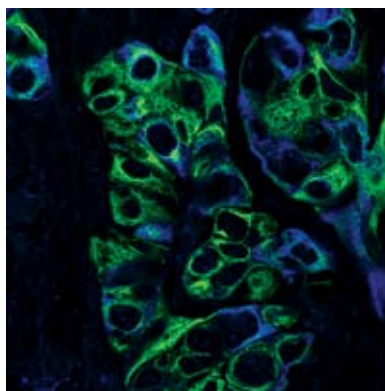
Specificity/Sensitivity: Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate) detects endogenous levels of ribosomal protein S6 only when phosphorylated at Ser235 and 236.

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. The antibody was conjugated to Alexa Fluor® 647 under optimal conditions with an F/P ratio of 2-5. The Alexa Fluor® 647 dye is maximally excited by red light (e.g. 633 nm He-Ne laser). Antibody conjugates of the Alexa Fluor® 647 dye produce bright far-red-fluorescence emission, with a peak at 665 nm.



Phospho-S6 Ribosomal Protein (Ser235/236) (Alexa Fluor® 647 Conjugate)

Flow cytometric analysis of K562 cells, untreated (green) or STI571-treated (blue), using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate).



Confocal immunofluorescent analysis of paraffin-embedded H3255 xenograft using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate) (blue) and Pan-Keratin (C11) Mouse mAb (Alexa Fluor® 488 Conjugate) #4523 (green).

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

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide, 2 mg/ml BSA. Store at 4°C. *Protect from light. Do not freeze.*

*Species cross-reactivity other than human is determined by western blot using the unconjugated antibody.

Recommended Antibody Dilutions:

Immunofluorescence (IF-IC)	1:50
Immunofluorescence (IF-P)	1:50
Flow Cytometry	1:50

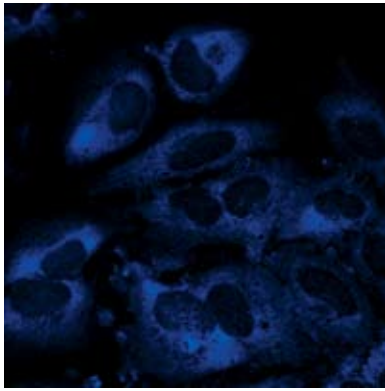
For application specific protocols please see the web page for this product at www.cellsignal.com.

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

The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents.

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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



Confocal immunofluorescent analysis of HeLa cells treated with 20% serum (left) or with Rapamycin, Wortmannin and U0126 inhibitors (right) using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate).

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.