**Phospho-S6 Ribosomal Protein (Ser235/236) (E2R1O) Mouse mAb**

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

**Applications**
- **W, IF-IC**

**Species Cross-Reactivity**
- H, M, R, Mk

**Molecular Wt.** 32 kDa

**Isotype**
- Mouse IgG2b

**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). Those 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).

**Background References:**

**Specificity/Sensitivity:** Phospho-S6 Ribosomal Protein (Ser235/236) (E2R10) Mouse mAb detects endogenous levels of S6 ribosomal protein only when phosphorylated at Ser235 and Ser236.

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

**Recommended Antibody Dilutions:**
- Western blotting: 1:1000
- Immunofluorescence (IF-IC): 1:400

**Fixative:** 4% Formaldehyde
**Permeabilization:** 0.3% Triton X-100

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

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

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- Western blotting: 1:1000
- Immunofluorescence (IF-IC): 1:400

**Fixative:** 4% Formaldehyde
**Permeabilization:** 0.3% Triton X-100

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

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

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Western blot analysis of extracts from SK-MEL-28 cells, untreated (-) or treated with λ-phosphatase (+), using Phospho-S6 Ribosomal Protein (Ser235/236) (E2R1O) Mouse mAb (upper) and S6 Ribosomal Protein (54D2) Mouse mAb #2317 (lower).

Confocal immunofluorescent analysis of HeLa cells, serum-starved overnight and then treated with Rapamycin #9904 (100 nM, 2 hr; left) or FBS (20%, 30 min; right), using Phospho-S6 Ribosomal Protein (Ser235/236) (E2R1O) Mouse mAb (green). Actin filaments were labeled with DyLight™ 650 Phalloidin #12956 (red). Blue = DAPI #4083 (fluorescent DNA dye).