

#9918 Store at -20°C

# Translational Control Sampler Kit

1 Kit  
 (6 x 40 µl)



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

rev. 03/23/10

**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.**

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-eIF2α (Ser51) (D9G8) XP™ Rabbit mAb	3398	40 µl	38 kDa	Rabbit IgG
Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb	4060	40 µl	60 kDa	Rabbit IgG
Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb	2855	40 µl	15-20 kDa	Rabbit IgG
Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb	4858	40 µl	32 kDa	Rabbit IgG
Phospho-p70 S6 Kinase (Thr389) (108D2) Rabbit mAb	9234	40 µl	70, 85 kDa	Rabbit IgG
Phospho-eIF4E (Ser209) Antibody	9741	40 µl	25 kDa	Rabbit IgG
LY294002 (PI3 Kinase Inhibitor)	9901	.3 mg	307 Da	
Rapamycin (FRAP/mTOR Inhibitor)	9904	2 µg	914.2 Da	
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The Translational Control Sampler Kit provides a fast and economical means of evaluating the activity of pathways regulating translation. The kit contains enough primary and secondary antibodies to perform four Western mini-blot experiments, as well as specific inhibitors of PI3 kinase and mTOR/FRAP.

**Background:** Key steps in translational control occur at the level of eukaryotic initiation factor 4F (eIF4F) and p70 S6 kinase regulation. eIF4F is a complex whose functions include the recognition of the mRNA 5' cap structure. Several stimuli, such as insulin and various growth and survival factors, regulate the eIF4F complex and p70 S6 kinase primarily by triggering a signaling cascade dependent on sequential activation of PI3K, Akt/PKB and mTOR/FRAP kinases. Akt is activated by phosphorylation within the C-terminus at Ser473 and within the activation loop at Thr308 by phospholipid-dependent kinases. Inactivation *in vivo* of PI3K by the highly selective inhibitor LY294002 inhibits Akt and downstream elements of this cascade. Direct phosphorylation of mTOR/FRAP at Ser2448 by Akt is a key regulatory event controlling its kinase activity. mTOR/FRAP activity can be effectively blocked by Rapamycin, leading to inactivation of eukaryotic initiation factor 4E binding protein 1 (4E-BP1), an inhibitor of translation initiation, and activation of p70 S6 kinases. Inactivation of 4E-BP1 by sequential phosphorylation causes the release of eIF4E, which, together with eIF4G and other factors, forms a functional eIF4F cap binding complex. p70 S6 kinases phosphorylates the 40S ribosomal subunit protein S6 and stimulates the translation of 5' oligopyrimidine tract containing mRNAs. The Erk pathway is also involved in regulation at this level by regulating the eIF4E kinase, Mnk1, and activating p70 S6 kinase. Tuberin, a product of the tumor suppressor gene

TSG2, is directly phosphorylated at Thr1462 by Akt/PKB. Tuberin inhibits the mammalian target of rapamycin, mTOR, which results in inhibition of p70 S6 kinase and activation of 4E-BP1 and, therefore, inhibition of translation.

**Specificity/Sensitivity:** Each phospho-specific antibody in the Translational Control Antibody Sampler Kit detects the intended target only when phosphorylated at the indicated site. Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb may cross-react with 4E-BP2 and 4E-BP3 when phosphorylated at equivalent sites.

**Source/Purification:** Phospho-specific polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser209 of human eIF4E. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Phospho-specific rabbit monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser473 of mouse Akt, Thr37 and Thr46 of mouse 4E-BP1, Ser51 of human eIF2α, Ser235 and Ser236 of human ribosomal protein S6, and Thr389 of human p70 S6 kinase.

**Directions for Inhibitor Use:** **LY294002 (PI3 Kinase Inhibitor):** For 10 mM stock, reconstitute 0.3 mg LY294002 in 98 µl DMSO. For 50 mM stock, reconstitute 0.3 mg in 20 µl DMSO. Store aliquots at -20°C. Recommended final treatment: 10 µM for one hour prior to, and for the duration of, the stimulation.

**Rapamycin (FRAP/mTOR Inhibitor):** For 100 µM stock, reconstitute 2 µg Rapamycin in 22 µl methanol. Recommended final treatment: 10 nM for 1 hour prior to stimulation.

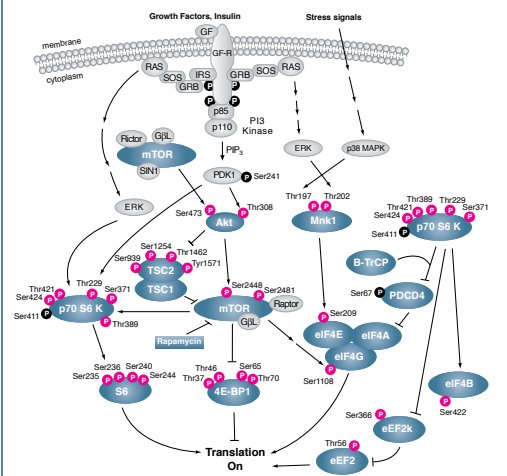
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

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

**Background References:**

- (1) Gingras, A. et al. (1999) *Annu. Rev. Biochem.* 68, 913–963.
- (2) Gingras, A.C. et al. (2001) *Genes and Develop.* 15, 807–826.
- (3) Dennis, P.B. et al. (1999) *Curr. Opin. Genet. Dev.* 9, 49–54.
- (4) Volarevic, S. and Thomas, G. (2000) *Prog. Nucleic Acid Res. Mol. Biol.* 65, 101–127.
- (5) Pyronnet, S. et al. (2000) *Biochem. Pharmacol.* 60, 1237–1243.
- (6) Dever, T.E. (2002) *Cell* 108, 545–556.
- (7) Goncharova, E. et al. (2002) *J. Biol. Chem.* 277, 30958–30967.

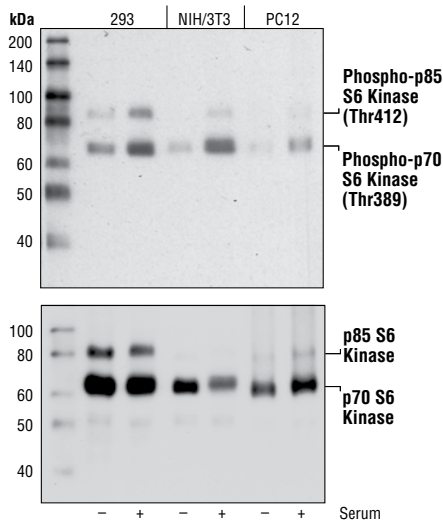


**Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U.S. Patents No. 5,675,063 and in some instances 7,429,487) from Eptomics, Inc.**

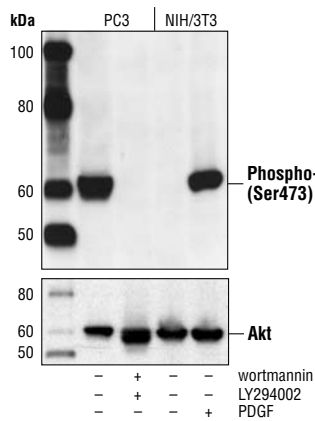
© 2010 Cell Signaling Technology, Inc.

**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.

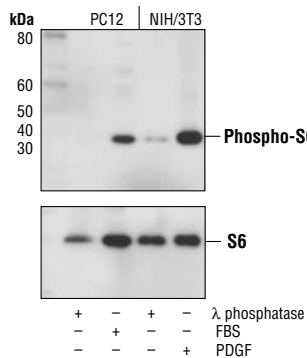
page 1 of 3



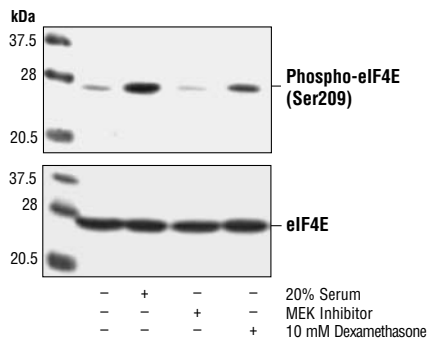
Western blot analysis of extracts from serum starved or serum-treated (20%) 293, NIH/3T3, and PC12 cells, using **Phospho-p70 S6 Kinase (Thr389) (108D2) Rabbit mAb #9234** (upper), or p70 S6 Kinase (49D7) Rabbit mAb #2708 (lower).



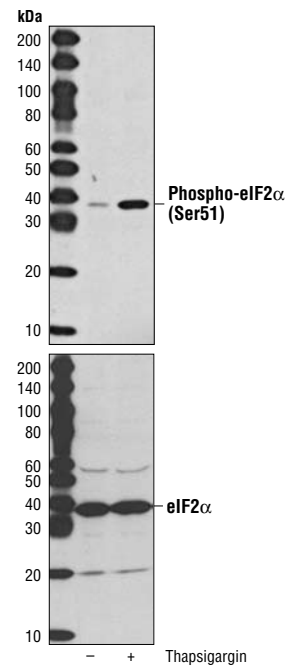
Western blot analysis of extracts from PC-3 cells, untreated or LY294002/wortmannin-treated, and NIH/3T3 cells, serum-starved or PDGF-treated, using **Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060** (upper) or Akt (pan) (C67E7) Rabbit mAb #4691 (lower).



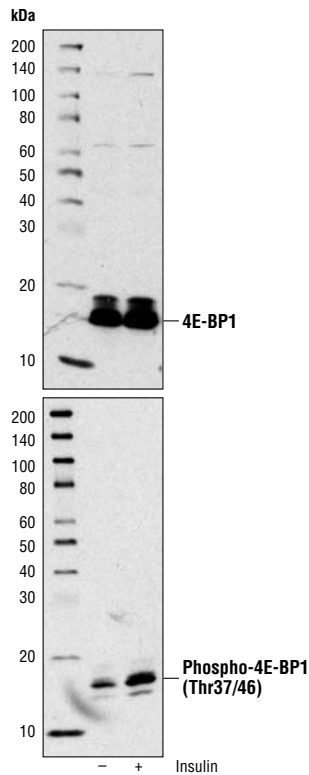
Western blot analysis of extracts from PC12 and NIH/3T3 cells, treated with  $\lambda$  phosphatase, 20% FBS (20 min) or 100 ng/ml PDGF (20 min) as indicated, using **Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP™ Rabbit mAb #4858** (upper) or S6 Ribosomal Protein (5G10) Rabbit mAb #2217 (lower).



Western blot analysis of extracts from NIH/3T3 cells, untreated or treated with serum, PD98059 or Dexamethasone, using **Phospho-eIF4E (Ser209) Antibody #9741** (upper) or eIF4E Antibody #9742 (lower).



Western blot analysis of extracts from C2C12 cells, untreated or thapsigargin-treated, using **Phospho-eIF2 $\alpha$  (Ser51) (D9G8) XP™ Rabbit mAb #3398** (upper) or eIF2 $\alpha$  Antibody #9722 (lower).



Western blot analysis of extracts from 293T cells using 4E-BP1 Antibody #9452 (upper) and **Phospho-4E-BP1 (Thr37/46) Antibody #2855** (lower). The cells were starved for 24 hours in serum-free medium and underwent a 1 hour amino acid deprivation. Amino acids were replenished for 1 hour. Cells were then either untreated (-) or treated with 100 nM insulin (+) for 30 minutes.

## Western Immunoblotting Protocol (Primary Antibody Incubation In BSA)

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.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.