Store at -20°C

#2903

# p70 S6 Kinase Substrates Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

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rev. 06/16

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

| Products Included | Product # | Quantity | Mol. Wt. | Isotype |
|---|---|---|---|---|
| Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb | 4858 | 20 µl | 32 kDa | Rabbit IgG |
| Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP® Rabbit mAb | 5364 | 20 µl | 32 kDa | Rabbit IgG |
| Phospho-eIF4B (Ser422) Antibody | 3591 | 20 µl | 80 kDa | Rabbit IgG |
| Phospho-eEF2k (Ser366) Antibody | 3691 | 20 µl | 105 kDa | Rabbit IgG |
| Phospho-p70 S6 Kinase (Thr389) (108D2) Rabbit mAb | 9234 | 20 µl | 70, 85 kDa | Rabbit IgG |
| p70 S6 Kinase (49D7) Rabbit mAb | 2708 | 20 µl | 70, 85 kDa | Rabbit IgG |
| Anti-rabbit IgG, HRP-linked Antibody | 7074 | 100 µl | | Goat |

**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 for validation data and a complete listing of recommended companion products.**



**See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.**

**Description:** The p70 S6 Kinase Substrates Antibody Sampler Kit provides a fast and economical means of evaluating several substrates of p70 S6 Kinase. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

**Background:** p70 S6 kinase is a mitogen activated Ser/Thr protein kinase that is required for cell growth and G1 cell cycle progression (1,2). p70 S6 kinase phosphorylates the S6 protein of the 40S ribosomal subunit and is involved in translational control of 5' oligopyrimidine tract mRNAs (1). Important S6 ribosomal protein phosphorylation sites include several residues (Ser235, Ser236, Ser240, Ser244) located wtihin a small, carboxy-terminal region of the S6 protein (3,4). p70 S6 kinase has been shown to phosphorylate eIF4B at the rapamycin-sensitive site Ser422 *in vivo*, and a Ser422Ala mutant of eIF4B shows diminished activity in an *in vitro* translation assay (5). Phosphorylation of eEF2K by p70 S6 kinase and p90RSK leads to inactivation of eEF2K (6), facilitating the dephosphorylation of eEF2 and thus promoting translation.

**Specificity/Sensitivity:** Each antibody in the p70 S6 Kinase Substrates Antibody Sampler Kit detects endogenous levels of its target protein. p70 S6 Kinase (49D7) Antibody #2708 also recognizes p85 S6 Kinase. Phospho-p70 S6 Kinase (Thr389) (108D2) Rabbit mAb #9234 also detects p85 S6 Kinase when phosphorylated at Thr412 and possibly S6KII when phosphorylated at Thr401. The other antibodies in the kit do not cross react with other proteins.

**Source/Purification:** Antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the amino-terminus of human p70 S6 Kinase; to synthetic phosphopeptides corresponding to residues surrounding Thr389 of human p70 S6 Kinase; Ser235 and 236 of human ribosomal protein; Ser240 and 244 of human ribosomal protein; Ser422 of human eIF4B; and Ser366 of human eEF2k. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

**Background References:**

(1) Pullen, N. and Thomas, G. (1997) *FEBS Lett* 410, 78–82.

(2) Dufner, A. and Thomas, G. (1999) *Exp Cell Res* 253, 100–9.

(3) Ferrari, S. et al. (1991) *J Biol Chem* 266, 22770–5.

(4) Flotow, H. and Thomas, G. (1992) *J Biol Chem* 267, 3074–8.

(5) Raught, B. et al. (2004) *EMBO J* 23, 1761–9.

(6) Wang, X. et al. (2001) *EMBO J* 20, 4370–9.



**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.

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# 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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

## C Membrane Blocking and Antibody Incubations

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

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

## D Detection of Proteins

1. 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.

2. 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.