

Phospho-(Ser) Kinase Substrate Antibody Sampler Kit

✓ 1 Kit
(4 x 40 µl)

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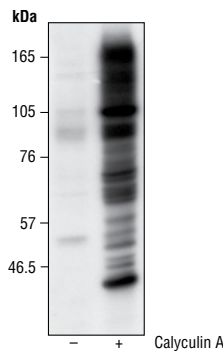
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Isotype
Phospho-(Ser) CDKs Substrate Antibody	2324	40 µl	Rabbit IgG
Phospho-MAPK/CDK Substrates (PXSP or SPXR/K) (34B2) Rabbit mAb	2325	40 µl	Rabbit IgG
Phospho-(Ser) Arg-X-Tyr/Phe-X-pSer Motif Antibody	2981	40 µl	Rabbit IgG
Phospho-(Ser) 14-3-3 Binding Motif (4E2) Mouse mAb	9606	40 µl	Mouse IgG1
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl	Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl	Goat

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

Description: Phospho-(Ser) Kinase Substrate Antibody Sampler Kit contains 40 µl of each polyclonal primary antibody [Phospho-(Ser) CDKs Substrate Antibody, Phospho-(Ser) Arg-X-Tyr/Phe-X-pSer Motif Antibody], 40 µl of Phospho-MAPK/CDK Substrates (PXSP or SPXR/K) (34B2) Rabbit mAb and 40 µl of Phospho-(Ser) 14-3-3 Binding Motif (4E2) Mouse mAb.

Background: Phospho-(Ser) kinases and phosphatases play critical roles in a wide range of biological processes. Each phospho-(Ser) kinase phosphorylates serine within a specific motif. The MAPK and CDK families of serine protein kinases phosphorylate serine followed by proline residue (1-3). The consensus amino acid sequence for CDK substrate is (K/R)(S*)PX(K/R), where X denotes any one of the 20 amino acids and S* is the phosphorylation site (4-6). MAPK phosphorylates substrates with the consensus sequence PX(S*)P. The 14-3-3 proteins are a highly conserved family of proteins involved in the regulation of cell survival, apoptosis, proliferation and checkpoint control (7-11). Binding of 14-3-3 is mediated through phospho-serine-containing proteins (12). Two different phospho-serine-containing motifs are found using a degenerate phospho-serine-oriented peptide library technique, RSXS*XP and RXY/FXS*XP (12). Motif 1 (Arg/Lys and Ser at positions -3 and -2, phospho-Ser at position 0, and Pro at position +2) is found in critical regulatory proteins including Bad, cdc25C, FoxO3A, PKC and c-Raf (11, 13). Motif 2 (RXY/FXS*XP) is found in critical regulatory proteins including cdc25A, cdc25B, PKCγ, IRS-1 and BCR (12). Although Phospho-(Ser) Arg-X-Tyr/Phe-X-pSer Motif Antibody binds 14-3-3 binding motif 2 with no requirement for proline in the +2 position, it provides a powerful tool for the discovery and characterization of potential 14-3-3 binding motif 2-containing proteins or other proteins with the RXY/FXS* motif. Antibodies specific to particular kinase substrates are



Western blot analysis of extracts from A431 cells, untreated or calyculin A treated using **Phospho-(Ser) Arg-X-Tyr/Phe-X-pSer Motif Antibody #2981**.

invaluable reagents in determining kinase activity and identifying potential new kinase substrates. CST has developed antibodies that recognize phosphorylated serine within the context of a protein motif that is phosphorylated by MAPK/CDK, CDKs or 14-3-3. As shown by DELFIA or ELISA, each phospho-(Ser) kinase substrate antibody in this sampler kit is specific to its kinase substrate motif.

Specificity/Sensitivity: Each antibody detects endogenous levels of phospho-(Ser) proteins of specific kinase substrate groups.

Phospho-(Ser) CDKs Substrate Antibody detects phospho-serine in a (K/R)(S*)PX(K/R) motif. The antibody is phospho-specific but does not recognize phospho-serine in the absence of the CDK motif. The antibody does not cross-react with phospho-threonine- or phospho-tyrosine-containing peptides/proteins.

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

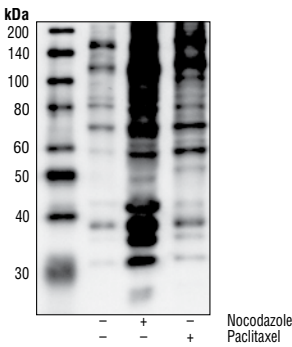
Please visit www.cellsignal.com for a complete listing of recommended companion products.

- Background References:**
- (1) Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol.* 200, 62–81.
 - (2) Karin, M. (1994) *Curr. Opin. Cell Biol.* 6, 415–424.
 - (3) Lewis, T.S. et al. (1998) *Adv. Cancer Res.* 74, 49–139.
 - (4) Songyang, Z. et al. (1996) *Mol. Cell Biol.* 16, 6486–6493.
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 - (6) Holmes, J.K. and Solomon, M.J. (1996) *J. Biol. Chem.* 271, 25240–25246.
 - (7) Aitken, A. (1995) *Trends Biochem. Sci.* 20, 95–97.
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 - (9) Pivnicka-Worms, H. (1999) *Nature* 401, 535, 537.
 - (10) Tzivion, G. et al. (1998) *Nature* 394, 88–92.
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 - (12) Muslin, A.J. et al. (1996) *Cell* 84, 889–897.
 - (13) Yaffe, M.B. et al. (1997) *Cell* 91, 961–971.

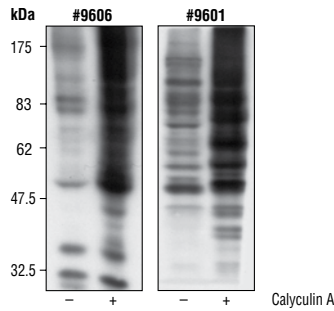
Rabbit monoclonal antibody is produced under license (granting certain rights including those under U. S. Patents No. 5,675,063 and 7,429,487) from Epitomics, Inc.

License/Use Restrictions: Use of CST Motif Antibodies within certain methods (e.g., U.S. Patent No.'s 7,198,896 & 7,300,753) may require a license from CST. For information regarding academic licensing terms please have your technology transfer office contact CST Legal Department at CST_ip@cellsignal.com. For information regarding commercial licensing terms please contact CST Pharma Services Department at ptmscan@cellsignal.com.

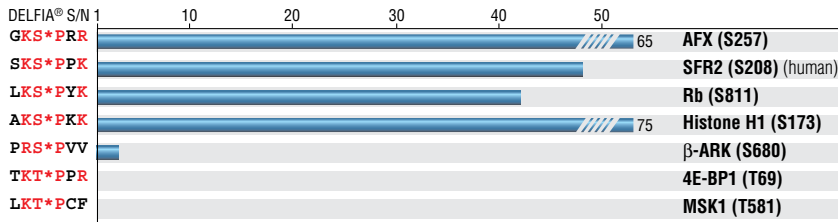
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA E-P—ELISA Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish B—bovine
Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.



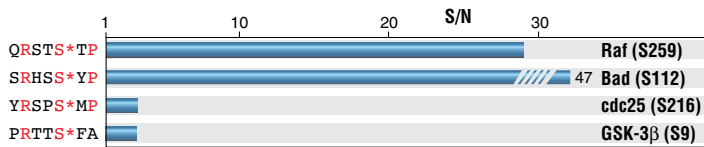
Western blot analysis of extracts from HeLa cells, untreated or treated with the microtubule destabilizing agents nocodazole or paclitaxol, using **Phospho-(Ser) CDKs Substrate Antibody #2324**.



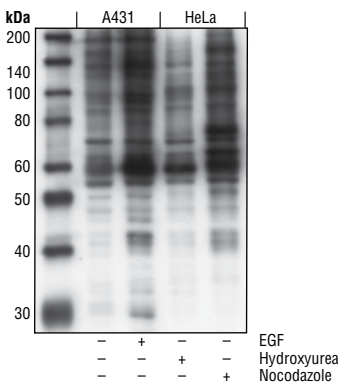
Western blot analysis of extracts from A431 cells, untreated or calyculin A-treated, using **Phospho-(Ser) 14-3-3 Binding Motif (4E2) Mouse mAb #9606** (left) or **Phospho-(Ser) 14-3-3 Binding Motif Antibody #9601** (right).



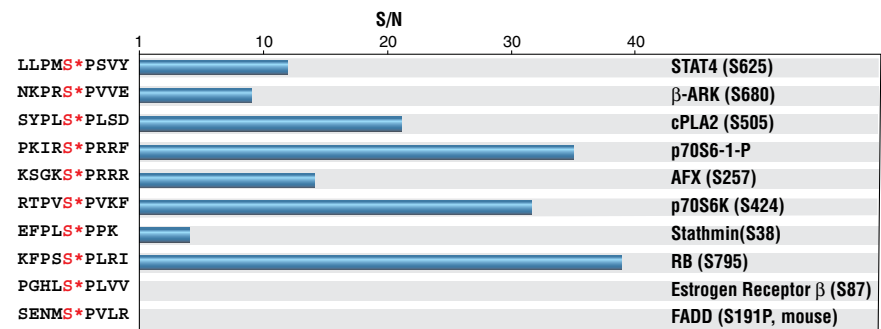
Phospho-(Ser) CDKs Substrate Antibody #2324 ELISA Assay: Signal-to-noise ratio of phospho- versus nonphospho-peptides. (S* denotes phosphorylated serine.)



Phospho-(Ser) 14-3-3 Binding Motif (4E2) Mouse mAb #9606 ELISA assay: Signal-to-noise ratio of phospho- versus nonphospho-14-3-3 binding motif peptides. (S* denotes phosphorylated serine.)



Western blot analysis of extracts from serum-starved A431 cells, untreated or treated in culture with EGF, and HeLa cells, untreated, hydroxyurea-treated (G1/S) or Nocodazole-treated (G2/M), using **Phospho-MAPK/CDK Substrate (PXS*P or S*PXR/K) (34B2) Rabbit mAb #2325**.



Phospho-MAPK/CDK Substrate (PXS*P or S*PXR/K) (34B2) Rabbit mAb #2325 ELISA Assay: Signal-to-noise ratio of phospho- versus nonphospho-peptides. (S* denotes phosphorylated serine.)

Specificity/Sensitivity (cont.):

Phospho-MAPK/CDK Substrates (PXS*P or S*PXR/K) (34B2) Rabbit mAb detects phospho-serine in a PXS*P or S*PXR/K motif, as well as a PXS*PXR/K motif. The antibody is phospho-specific, and does not react with phospho-threonine- or phospho-tyrosine-containing peptides/proteins.

Phospho-(Ser) Arg-X-Tyr/Phe-X-pSer Motif Antibody detects endogenous levels of proteins containing the Arg-X-Tyr/Phe-X-pSer motif. This antibody does not cross-react with nonphosphorylated serine or phospho-threonine proteins with the same motif or other phospho-serine/threonine-containing proteins and peptides without this motif.

Phospho-(Ser) 14-3-3 Binding Motif (4E2) Mouse mAb binds peptides and proteins containing phospho-Ser surrounded by Pro at the +2 position and Arg/Lys at the -3 position. By ELISA, the antibody recognizes a wide range of peptides containing this phosphorylated 14-3-3 binding motif in a manner that is phospho-specific and largely independent of the surrounding amino acid sequence. The antibody weakly cross-reacts with sequences containing phospho-Thr instead of phospho-Ser in this motif, and with sequences containing phospho-Ser surrounded by Phe at the +1 position and Arg/Lys at the -3 position. No cross-reactivity is observed with corresponding nonphosphorylated sequences or with other phospho-Ser/Thr/Tyr containing motifs. Phospho-(Ser) 14-3-3 Binding Motif (4E2) Mouse mAb complements our polyclonal Phospho-(Ser) 14-3-3 Binding Motif Antibody #9601 by showing slightly different and overlapping specificity.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with phospho-peptides containing the kinase substrate motif and purified by protein A and peptide affinity chromatography.

Rabbit monoclonal antibody is produced by immunizing rabbits with synthetic phospho-MAPK/CDK substrate peptides.

Mouse monoclonal antibody is produced by immunizing mice with phospho-(Ser) 14-3-3 binding motif peptides (KLH-coupled).

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 cm²) 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.