

Phospho-(Ser/Thr) Kinase Substrate Antibody Sampler Kit

✓ 1 Kit
 (6 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-(Thr) MAPK/CDK Substrate Mouse mAb	2321	40 µl	Mouse IgM
Phospho-Akt Substrate (RXXXS/T) (110B7E) Rabbit mAb	9614	40 µl	Rabbit IgG
Phospho-PKA Substrate (RRXS/T) (100G7E) Rabbit mAb	9624	40 µl	Rabbit IgG
Phospho-(Ser/Thr) ATM/ATR Substrate Antibody	2851	40 µl	Rabbit IgG
Phospho-(Ser) PKC Substrate Antibody	2261	40 µl	Rabbit IgG
Phospho-(Ser) CDKs Substrate Antibody	2324	40 µl	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl	Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl	Horse

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

Description: Phospho-(Ser/Thr) Kinase Substrate Antibody Sampler Kit contains 40 µl of each polyclonal primary antibody [Phospho-(Ser) PKC Substrate Antibody, Phospho-(Ser) CDKs Substrate Antibody and Phospho-(Ser/Thr) ATM/ATR Substrate Antibody], 40 µl each of Phospho-(Ser/Thr) Akt Monoclonal Substrate Antibody and Phospho-(Ser/Thr) PKA Substrate Antibody and 40 µl of Phospho-(Thr) MAPK/CDK Substrate mAb.

Background: Phospho-(Ser/Thr) kinases and phosphatases play critical roles in a wide range of biological processes. Each phospho-(Ser/Thr) kinase phosphorylates serine or threonine within a specific motif. Akt phosphorylates substrates at a serine or threonine only in a conserved motif characterized by arginine at positions -5 and -3 (1). Conventional PKC isozymes phosphorylate substrates containing serine or threonine, with arginine or lysine at the -3, -2 and +2 positions, and a hydrophobic amino acid at position +1 (2,3). A consensus phosphorylation site of PKA is serine or threonine with arginine at the -2 and -3 positions (3). The MAPK and CDK families of serine/threonine protein kinases phosphorylate threonine or serine followed by proline residue (3-5). The consensus amino acid sequence for CDK substrate is (K/R)(S*)PX(K/R), where denotes any one of the 20 amino acids and S* is the phosphorylation site (6-8). ATM and the related kinase ATR phosphorylate serine or threonine in an S*/T*Q motif (9,10).

Antibodies specific to particular kinase substrates are invaluable reagents in determining kinase activity and identifying potential new kinase substrates. CST has developed antibodies that recognize phosphorylated serine or threonine within the context of a protein motif that is phosphorylated by Akt, PKC, PKA, MAPK/CDK, CDKs or ATM/ATR. As shown by peptide pairing ELISA, each phospho-(Ser/Thr) kinase substrate antibody in this sampler kit is specific to its kinase substrate motif.

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

Phospho-(Ser/Thr) Akt Substrate (110B7E) Rabbit mAb preferentially recognizes peptides and proteins containing phospho-serine/threonine preceded by arginine at positions -5 and -3, in a manner largely independent of the surrounding amino acid sequence. Some cross-reactivity is observed for peptides that contain phospho-serine/threonine preceded by arginine at position -3. No cross-reactivity is observed with the corresponding nonphosphorylated sequences or with other phospho-serine/threonine-containing motifs. By ELISA, the antibody recognizes a wide range of peptides with phospho-threonine or Phospho-serine with arginine at -3 and -5 position.

Phospho-(Ser) PKC Substrate Antibody detects endogenous levels of many cellular proteins only when phosphorylated at serine residues surrounded by Arg or Lys at the -2 and +2 positions and a hydrophobic residue at the +1 position. The antibody does not cross-react with nonphosphorylated serine residues, with phospho-threonine in the same motif, or with phospho-serine in other motifs.

Phospho-PKA Substrate (RRXS/T*) (100G7E) Rabbit mAb* detects peptides and proteins containing a phospho-Ser/Thr residue with arginine at the -3 and -2 positions. It is a useful tool in identifying new substrates of PKA. The antibody recognizes other -3 arginine-bearing phospho-Ser/Thr peptides, such as substrate motifs for Akt and PKC, to a lesser extent. It does not recognize the nonphosphorylated substrate motif peptides.

Phospho-(Thr) MAPK/CDK Substrate Mouse mAb detects phospho-threonine only when followed by proline. It reacts with proteins/peptides phosphorylated on the Thr-Pro motif in an otherwise highly context-independent fashion. The

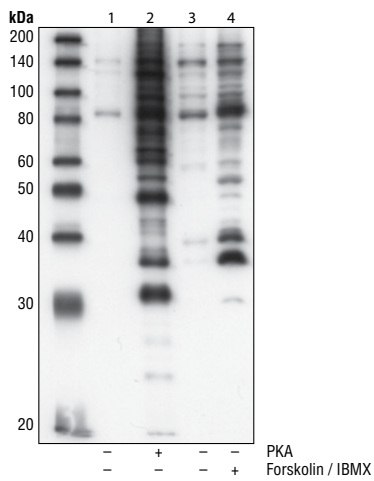
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

Recommended Antibody Dilutions:
 Western blotting 1:1000

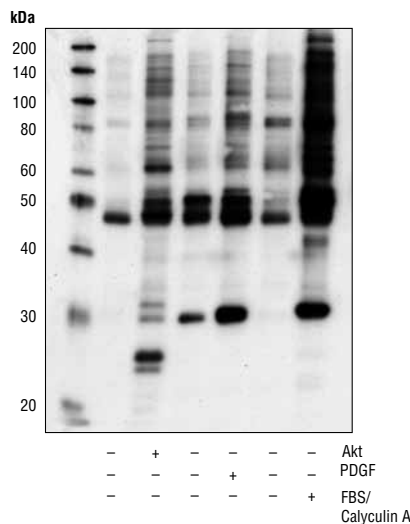
Please visit www.cellsignal.com for a complete listing of recommended companion products.

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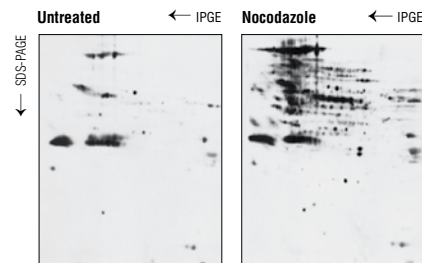
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine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



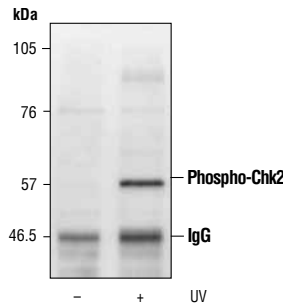
Western blot analysis of extracts from serum-starved A431 cells, phosphorylated *in vitro* with PKA kinase or treated in culture with forskolin/IBMX, using **Phospho-PKA Substrate (RRXS*T*) (100G7E) Rabbit mAb #9624**. Lysis buffer: 1.0% Triton X-100 (lanes 1 and 2), 2.0% SDS (lanes 3 and 4).



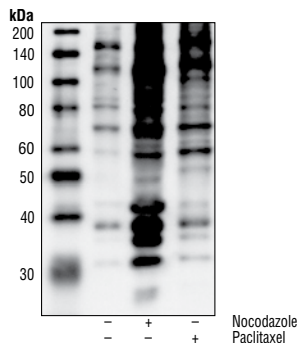
Western blot analysis of extracts from serum-starved NIH/3T3 cells, phosphorylated *in vitro* with Akt kinase, or treated in culture with PDGF or FBS/Calyculin A, using **Phospho-(Ser/Thr) Substrate (110B7E) Rabbit mAb #9614**.



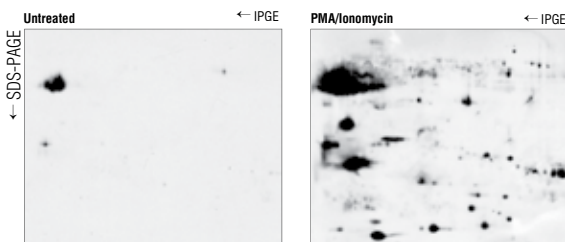
Western blot analysis of whole cell lysates from Jurkat cells untreated and treated with 1 µg/ml nocodazole for 12 hours prior to lysis, using **Phospho-(Thr) MAPK/CDK Substrate mAb #2321**. Proteins were separated by 2D electrophoresis prior to blotting.



Chk2 transfected and UV treated COS cell extracts immunoprecipitated with Chk2 antibody then detected by Western blotting using **Phospho-(Ser/Thr) ATM/ATR Substrate Antibody #2851**.



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



Western blot analysis of whole cell lysates of Jurkat cells untreated and treated with PMA (50 ng/ml) and ionomycin (1 µM) for 20 minutes prior to lysis. Proteins were separated by 2D electrophoresis prior to blotting and probed with **Phospho-(Ser) PKC Substrate Antibody #2261**.

antibody does not cross-react with phospho-threonine in the absence of an adjacent proline. The antibody does not cross-react with phospho-tyrosine, but does react with some phospho-serine peptides containing the phospho-serine-proline motif (e.g., phospho-Elk-1).

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.

Phospho-(Ser/Thr) ATM/ATR Substrate Antibody detects endogenous levels of proteins containing the ATM/ATR substrate motif. This antibody preferentially binds peptides and proteins that contain phospho-Ser/Thr preceded by Leu or similar hydrophobic amino acids at the -1 position and followed by Gln at the +1 position. The antibody does not cross-react with corresponding nonphosphorylated sequences or with other phospho-Ser/Thr-containing motifs.

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 antibodies are produced by immunizing rabbits with synthetic phospho-peptides (KLH-coupled).

Mouse monoclonal antibody (isotype: IgM) is produced by immunizing mice with a phospho-(Thr) MAPK/CDK substrate motif-containing peptide and is purified by protein A chromatography.

Background References:

- (1) Alessi, D.R. et al. (1996) *FEBS Lett.* 399, 333–338.
- (2) Nishikawa, K. et al. (1997) *J. Biol. Chem.* 272, 952–960.
- (3) Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol.* 200, 62–81.
- (4) Karin, M. (1994) *Curr. Opin. Cell Biol.* 6, 415–424.
- (5) Lewis, T.S. et al. (1998) *Adv. Cancer Res.* 74, 49–139.
- (6) Songyang, Z. et al. (1996) *Mol. Cell Biol.* 16, 6486–6493.
- (7) Songyang, Z. (1999) *Prog. Biophys. Mol. Biol.* 71, 359–372.
- (8) Holmes, J.K. and Solomon, M.J. (1996) *J. Biol. Chem.* 271, 25240–25246.
- (9) Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179–186.
- (10) Zhao, H. and Piwnicka-Worms, H. (2001) *Mol. Cell Biol.* 21, 4129–4139.

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.