Store at -20°C

#9920

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

☑ 1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Isotype
Phospho-(Ser/Thr) AMPK Substrate (P-S/T2-102) Rabbit mAb	5759	20 µl	Rabbit IgG
Phospho-Akt Substrate (RXRXXS/T) (110B7E) Rabbit mAb	9614	20 µl	Rabbit IgG
Phospho-PKA Substrate (RRXS/T) (100G7E) Rabbit mAb	9624	20 µl	Rabbit IgG
Phospho-(Ser/Thr) ATM/ATR Substrate (S*/T*QG) (P-S/T^2-100) Rabbit mAb	6966	20 µl	Rabbit IgG
Phospho-(Ser) PKC Substrate (P-S^3-101) Rabbit mAb	6967	20 µl	Rabbit IgG
Phospho-(Ser) CDKs Substrate (P-S^2-100) Rabbit mAb	9477	20 µl	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl	Goat

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

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.

Description: The Phospho-(Ser/Thr) Kinase Substrate Antibody Sampler Kit provides an economical means to investigate the downstream activity of select serine/threonine kinases. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

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). AMPK phosphorylates consensus motif (L/M)XRXX(S/T)XXXL (6). Antibodies recognizing the LXRXX(S/T) motif are very useful in the identification of AMPK substrates. 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 (4-6). ATM and the related kinase ATR phosphorylate serine or threonine in an S*/T*Q motif (7,8).

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: 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 (P-S^3-101) Rabbit mAb detects endogenous levels of many cellular proteins only when phosphorylated at Ser residues surrounded by Arg or Lys at the -2 and +2 positions. The antibody may also cross-react with S*X(R/K) motif-containing proteins. The antibody does not cross-react with nonphosphorylated Ser residues, phospho-Thr in the same motif, or phospho-Ser 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-(Ser/Thr) AMPK Substrate (P-S/T^2-102) Rabbit mAb preferentially recognizes endogenous proteins and peptides bearing the LXRXXpS/pT motif. The antibody also cross-reacts with proteins and peptides that only harbor an RXXpS/pT motif.

Phospho-(Ser) CDKs Substrate (P-S^2-100) Rabbit mAb recognizes phospho-serine in a KS*P motif. The antibody does not cross-react with phospho-threonine or phospho-tyrosine containing peptides/proteins.

Phospho-(Ser/Thr) ATM/ATR Substrate (S*/T*QG) (P-S/T^2-100) Rabbit mAb recognizes proteins containing phospho-Ser or phospho-Thr followed by Gln and Gly residues. To some extent, this antibody also recongizes proteins with an S*/T*Q motif.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides .

Background References:

(1) Alessi, D.R. et al. (1996) *FEBS Lett* 399, 333-8.
(2) Nishikawa, K. et al. (1997) *J Biol Chem* 272, 952-60.
(3) Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol* 200, 62-81.
(4) Songyang, Z. et al. (1996) *Mol Cell Biol* 16, 6486-93.
(5) Songyang, Z. (1999) *Prog Biophys Mol Biol* 71, 359-72.
(6) Holmes, J.K. and Solomon, M.J. (1996) *J Biol Chem* 271, 25240-6.
(7) Kastan, M.B. and Lim, D.S. (2000) *Nat Rev Mol Cell Biol* 1, 179-86.
(8) Zhao, H. and Piwnica-Worms, H. (2001) *Mol Cell Biol* 21, 4129-39.

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.

#9920

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.