Store at -20°C

#9615

Phospho-(Ser) Kinase Substrate Antibody Sampler Kit

☑ 1 Kit
(4 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) CDKs Substrate (P-S²-100) Rabbit mAb	9477	20 µl	Rabbit IgG
Phospho-MAPK/CDK Substrates (PXSP or SPXR/K) (34B2) Rabbit mAb	2325	20 µl	Rabbit IgG
Phospho-(Ser) Arg-X-Tyr/Phe-X-pSer Motif Antibody	2981	20 µl	Rabbit IgG
Phospho-(Ser) 14-3-3 Binding Motif (4E2) Mouse mAb	9606	20 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 20 µl of each polyclonal primary antibody [Phospho-(Ser) CDKs Substrate (P-S²-100) Rabbit mAb, Phospho-(Ser) Arg-X-Tyr/Phe-X-pSer Motif Antibody], 20 µl of Phospho-MAPK/CDK Substrates (PXSP or SPXR/K) (34B2) Rabbit mAb and 20 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 concensus 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 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 (P-S²-100) 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

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

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.

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.
(5) Songyang, Z. (1999) *Prog. Biophys. Mol. Biol.* 71, 359–372.
(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.
(8) Zha, J. et al. (1996) *Cell* 87, 619–628.
(9) Piwnica-Worms, H. (1999) *Nature* 401, 535, 537.
(10) Tzivion, G. et al. (1998) *Nature* 394, 88–92.
(11) Xing, H. et al. (2000) *EMBO J.* 19, 349–358.
(12) Muslin, A.J. et al. (1996) *Cell* 84, 889–897.
(13) Yaffe, M.B. et al. (1997) *Cell* 91, 961–971.

U.S. Patent No. 5,675,063

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.

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

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.