Phospho-(Ser/Thr) PKA Substrate Antibody



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

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

Applications	Species Cross-Reactivity*	Source	Motif
W, IP, IHC-P, E-P Endogenous	All	Rabbit**	(K/R)(K/R)X(S*/T*)

Background: An important class of kinases, refered to as Arg-directed kinases or AGC-family kinases, includes cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), protein kinase C, Akt and RSK. These kinases share a substrate specificity characterized by Arg at position -3 relative to the phosphorylated Ser or Thr (1,2). Phospho-PKA substrate-specific antibodies from Cell Signaling Technology are powerful tools for investigating the regulation of phosphorylation by PKA and other Arg-directed kinases, as well as for high throughput kinase drug discovery.

Specificity/Sensitivity: Phospho-(Ser/Thr) PKA Substrate Antibody detects peptides and proteins containing a phospho-serine/threonine residue with arginine at the -3 position. It is a useful tool in identifying substrates of AGC family kinases, including PKA and PKC. It does not crossreact with the nonphosphorylated PKA substrate motif. (U.S. Patent No's.: 6,441,140; 6,982,318; 7,259,022; 7,344,714; U.S.S.N. 11,484,485; and all foreign equivalents.)

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-PKA substrate peptide. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from A431 cells, phosphorylated in vitro by protein kinase A, Erk2 or cdc2/cyclin A, plus or minus PKA inhibitor (PKI), using Phospho-(Ser/Thr) PKA Substrate Antibody.

Background References:

- (1) Montminy, M. (1997) Annu Rev Biochem 66, 807-22.
- (2) Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol* 200, 62–81.



Phospho-(Ser/Thr) PKA Substrate Antibody ELISA Assay: Signal-to-noise ratio of phospho- versus nonphospho-peptides. (T* and S* denote phosphorylated threonine and serine.)

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.

 IMPORTANT: 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.

 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—zebrafish
 B—bovine

 Dg—dog
 Pg—pig
 Sc—S. cerevisiae
 Cerevisiae
 Hm—horse
 AII—all species expected
 Species enclosed in parentheses are predicted to react based on 100% homology.

*Species cross-reactivity is determined by western blot. **Anti-rabbit secondary antibodies must be used to detect this antibody.

aliquot the antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin) Unmasking buffer: SignalStain® Citrate (10X) #14746 Antibody diluent: SignalStain® Antil Detection reagent: SignalStain® Boost (†Optimal IHC dilutions determined using Detection Reagent.	1:200† Unmasking Solution body Diluent #8112 (HRP, Rabbit) #8114 SignalStain® Boost IHC
ELISA-Peptide	1:1000
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For application specific protocols please see the web page for this product at www.cellsignal.com.

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Immunohistochemical analysis of paraffin-embedded human breast carcinoma control (left) or λ phosphatase-treated (right), using Phsopho-(Ser/Thr) PKA Substrate Antibody.

Western blot analysis of extracts from A431 cells, untreated or calyculin A-treated, using Phospho-(Ser/Thr) PKA Substrate Antibody.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma, using Phospho-(Ser/Thr) PKA Substrate Antibody.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma, using Phospho-(Ser/Thr) PKA Substrate Antibody.