Revision 1

#9611 Store at -20C

Phospho-(Ser/Thr) Akt Substrate Antibody

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	Source:
WB, IP, IHC-P, E-P	All	Endogenous	Rabbit

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:500
Peptide ELISA (DELFIA)	1:500

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 antibody.

Specificity / Sensitivity

Phospho-(Ser/Thr) Akt Substrate Antibody preferentially recognizes peptides and proteins containing phospho-Ser/Thr preceded by Lys/Arg at positions -5 and -3, in a manner largely independent of other surrounding amino acids. Some cross-reactivity is observed for peptides that contain phospho-Ser/Thr preceded by Arg/Lys at positions -3 and -2. No cross-reactivity is observed with the corresponding nonphosphorylated sequences or with other phospho-Ser/Thr-containing motifs. By ELISA, the antibody recognizes a wide range of phosphorylated Akt substrate peptides, and, by 2-D gel Western blot analysis, it recognizes a large number of proteins presumed to be Akt substrates.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with phospho-Akt substrate peptides . Antibodies are purified by protein A and peptide affinity chromatography.

Background

An important class of kinases, referred 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). Akt plays a central role in mediating critical cellular responses including cell growth and survival, angiogenesis, and transcriptional regulation (3-5). While a number of Akt substrates are known (such as GSK-3, Bad, and caspase-9) many important substrates await discovery. Akt phosphorylates substrates only at Ser/Thr in a conserved motif characterized by Arg at positions -5 and -3 (6). Phospho-Akt substrate-specific antibodies from Cell Signaling Technology are powerful tools for investigating the regulation of phosphorylation by Akt and other Arg-directed kinases, as well as for high throughput kinase drug discovery.

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.
3. Marte, B.M. and Downward, J. (1997) *Trends Biochem Sci* 22, 355-8.
4. Jiang, B.H. et al. (2000) *Proc Natl Acad Sci USA* 97, 1749-53.
5. Scheid, M.P. and Woodgett, J.R. (2000) *Curr Biol* 10, R191-4.
6. Alessi, D.R. et al. (1996) *FEBS Lett* 399, 333-8.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin)
E-P: Peptide ELISA (DELFIA)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster
X: Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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