

Phospho-Akt Substrate (RXXS*/T*) (110B7E) Rabbit mAb (Magnetic Bead Conjugate)



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

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info@cellsignal.com

Web ■ www.cellsignal.com

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

Applications IP Endogenous	Species Cross-Reactivity All	Isotype Rabbit IgG
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Description: This Cell Signaling Technology antibody is immobilized by the covalent reaction of formylbenzamide-modified antibody with hydrazide-activated magnetic bead.

Phospho-Akt Substrate (RXXS*/T*) (110B7E) Rabbit mAb (Magnetic Bead Conjugate) is useful for immunoprecipitation of phosphorylated Akt substrate proteins.

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 CST 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.

Specificity/Sensitivity: Phospho-Akt Substrate (RXXS*/T*) (110B7E) Rabbit mAb (Magnetic Bead Conjugate) recognizes peptides and proteins containing phospho-Ser/Thr preceded by Arg at the -3 position. There is some preference observed for peptides that contain phospho-Ser/Thr preceded by Arg at both positions -5 and -3. (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: Monoclonal antibody is produced by immunizing animals with synthetic phospho-Akt substrate peptides.

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.

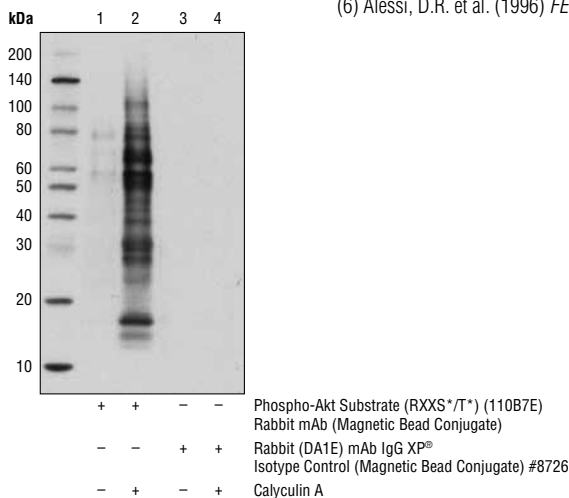
Storage: Supplied in PBS Buffer (pH 7.2), 0.1% Tween®20. Store at 4°C. *Do not aliquot the antibody.*

Directions for Use: Add 10 µl of well-vortexed beads to 200 µl of cell lysate at 1 mg/ml in 1X Cell Lysis Buffer (10X) #9803. See protocol for more details.

For bead washing and subsequent elution of immunocomplexes, the beads can be separated from solution using the 6-Tube Magnetic Separation Rack #7017. Place the tubes containing the beads in the 6-Tube Magnetic Separation Rack and wait 1 to 2 minutes for the solution to clear before carefully removing the supernatant. Remove the tubes from the 6-Tube Magnetic Separation Rack, add new solution, and resuspend the beads by gently vortexing or rocking the tube.

For application specific protocols please see the web page for this product at www.cellsignal.com.

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Immunoprecipitation of Jurkat cell lysates, untreated or treated with Calyculin A, using Phospho-Akt Substrate (RXXS*/T*) (110B7E) Rabbit mAb (Magnetic Bead Conjugate) (lane 1 and 2) and Rabbit (DA1E) mAb IgG XP® Isotype Control (Magnetic Bead Conjugate) #8726 (lane 3 and 4). The western blot was probed using Phospho-Akt Substrate (RXXS*/T*) (110B7E) Rabbit mAb (HRP Conjugate) #6950.

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 Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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