

# Phospho-Akt Substrate (RXXS\*/T\*) (110B7E) Rabbit mAb



**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
**Web** ■ www.cellsignal.com

rev. 04/22/16

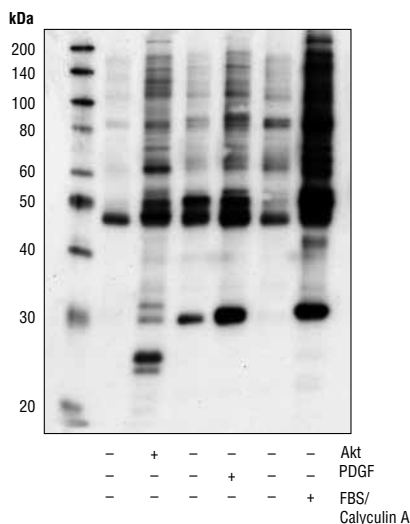
**For Research Use Only. Not For Use In Diagnostic Procedures.**

Applications	Species Cross-Reactivity*	Motif	Isotype
W, IP, IHC-P, E-P	All	RXXS*/T*	Rabbit IgG**

**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 including GSK-3, Bad and caspase-9 are known, many important substrates await discovery. Akt phosphorylates substrates only at serine/threonine in a conserved motif characterized by arginine 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.

**Specificity/Sensitivity:** Phospho-(Ser/Thr) Akt Substrate Motif (RXXS\*/T\*)(110B7) mAb recognizes peptides and proteins containing phospho-serine/threonine preceded by arginine at the -3 position. There is some preference observed for peptides that contain phospho-serine/threonine preceded by arginine 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.



Western blot analysis of extracts from serum-starved NIH/3T3 cells, phosphorylated in vitro with Akt kinase, or treated in culture with PDGF or FBS/Calyculin A, using Phospho-Akt Substrate (RXXS\*/T\*) (110B7E) Rabbit mAb.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

\*Species cross-reactivity is determined by western blot.

\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

**Recommended Antibody Dilutions:**

Western blotting 1:1000  
Immunoprecipitation 1:50  
Immunohistochemistry (Paraffin) 1:1000†  
Unmasking buffer: Citrate  
Antibody diluent: SignalStain® Antibody Diluent #8112  
Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114

†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

ELISA-Peptide 1:1000

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**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.

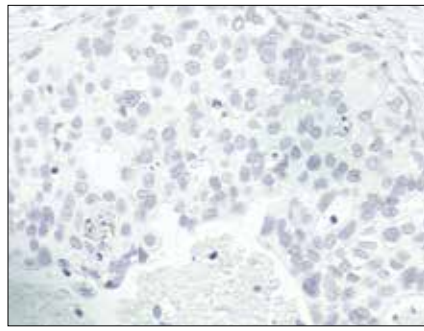
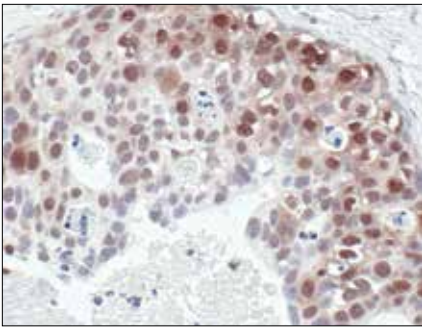
U.S. Patent No. 5,675,063

Tween®20 is a registered trademark of ICI Americas, Inc.

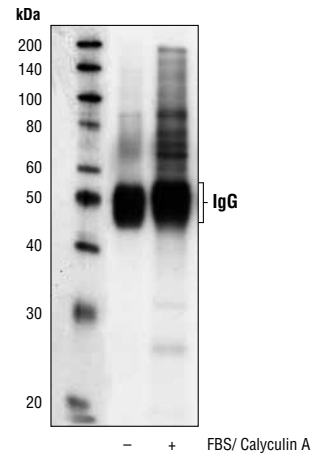
**License/Use Restrictions:** Use of CST Motif Antibodies within certain methods (e.g., U.S. Patent No.'s 7,198,896 & 7,300,753) may require a license from CST. For information regarding academic licensing terms please have your technology transfer office contact CST Legal Department at [CST\\_ip@cellsignal.com](mailto:CST_ip@cellsignal.com). For information regarding commercial licensing terms please contact CST Pharma Services Department at [ptmscan@cellsignal.com](mailto: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.**

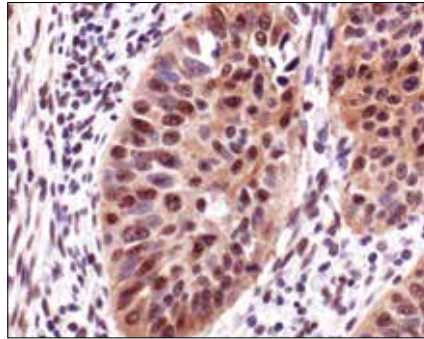
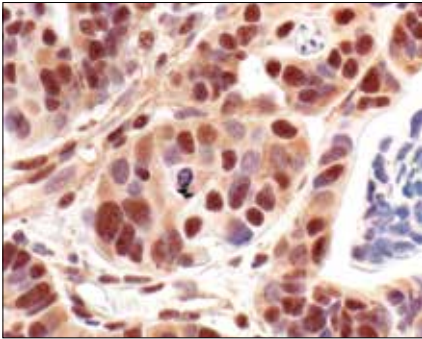
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish 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.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma control (left) or  $\lambda$  phosphatase-treated (right), using Phospho-Akt Substrate (RXXS/T) (110B7E) Rabbit mAb.

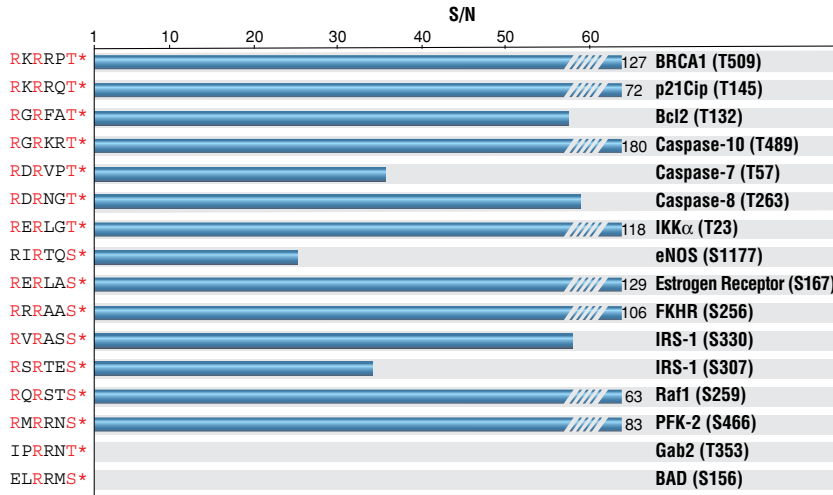


Immunoprecipitation of extracts from serum-starved NIH/3T3 cells, untreated or treated with FBS/Calyculin A, using Phospho-Akt Substrate (RXXS/T) (110B7E) Rabbit mAb, followed by Western blot analysis using the same antibody.



Immunohistochemical analysis of paraffin-embedded renal cell carcinoma, using Phospho-Akt Substrate (RXXS/T) (110B7E) Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded lung carcinoma, using Phospho-Akt Substrate (RXXS/T) (110B7E) Rabbit mAb.



Phospho-Akt Substrate (RXXS/T) (110B7E) Rabbit mAb ELISA Assay: Signal-to-noise ratio of phospho- versus nonphospho-peptides. (T\* and S\* denote phosphorylated threonine and serine.)