

#9381 Store at -20°C

Phospho-Threonine Antibody (P-Thr-Polyclonal)

✓ 100 µl (10 Western mini-blot)



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

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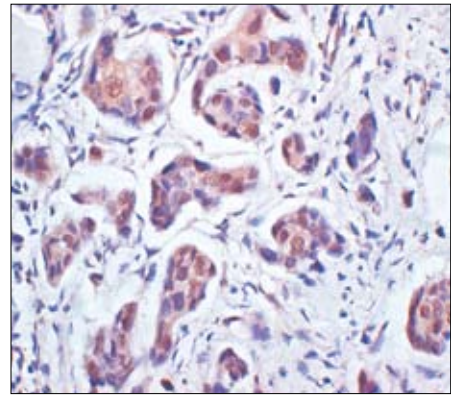
This product is for *in vitro* research use only and is not intended for use in humans or animals. This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications	Species Cross-Reactivity*	Source
W, IP, IHC-P, E-P Endogenous	All	Rabbit**

Background: Much of the dynamic behavior of cellular proteins, including the regulation of molecular interactions (1), subcellular localization (2) and transcriptional regulation (3) is controlled by a variety of posttranslational modifications (4). Antibodies specific for these posttranslational modifications are invaluable tools in the quest to understand normal and pathogenic molecular and cellular behavior.

General protein modification antibodies are designed to react with modified amino acid residues (e.g., phospho-threonine, phospho-tyrosine, acetyl-lysine, nitro-tyrosine) independently of the sequence in which they are embedded. This ability to recognize modified residues in a "context independent" fashion gives these antibodies broad reactivities, presumably conferring upon them the ability to react with hundreds of distinct proteins. This broad pattern of reactivity makes these antibodies especially valuable in multiplex analyses and target discovery programs.

Protein kinases are among the most abundant eukaryotic regulatory proteins; over 500 separate kinase genes are encoded in mammalian genomes (5,6). In spite of the importance of kinases in eukaryotic biology, relatively few of their physiological targets are known. Phospho-Threonine Antibody (P-Thr-Polyclonal) #9381 and Phospho-Threonine (42H4) mAb #9386 provide powerful tools for discovering targets of serine/threonine kinases, for monitoring and characterizing *in vitro* threonine phosphorylation reactions as well as for high throughput Ser/Thr kinase drug discovery.

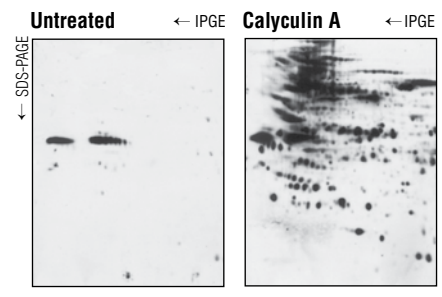


Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing staining of proteins with phosphorylated threonine residues, using Phospho-Threonine Antibody (P-Thr-Polyclonal) #9381.

Specificity/Sensitivity: Phospho-Threonine Antibody (P-Thr-Polyclonal) detects proteins and peptides phosphorylated at threonine residues in a manner largely independent of the surrounding amino acid sequence. The antibody is phospho-specific and may cross-react with some phospho-tyrosine- or phospho-serine-containing sequences. By ELISA, it recognizes a wide variety of threonine-phosphorylated peptides, and by 2D gel Western blot analysis, it recognizes a large number of presumably threonine-phosphorylated proteins. CST recommends the use of Phospho-Threonine-Proline mAb (p-Thr-Pro-101) #9391 to detect proteins containing threonine followed by proline. (Patented, U.S. No. 6,441,140 and Patents Pending.)

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic phospho-Thr-containing peptides KLH-coupled. Antibodies are purified by protein A and peptide affinity chromatography.

License/Use Restrictions: The purchase of this product does not include the right or license to practice any technology or methods described and claimed in U.S. Patent Number 7,198,896 and foreign equivalents, "Immunoaffinity Isolation of Modified Peptides from Complex Mixtures", Rush et al., Pub. Date March 6, 2003 (and equivalents). Use of purchased product in such methods, in any field, requires a separate license from Cell Signaling Technology, Inc. A limited license to use such methods may, however, be obtained by non-profit entities via purchase of any PhosphoScan® Kit provided that such limited license extends only to the use of the particular antibody sold within that Kit.



Western blot analysis of extracts from Jurkat cells, untreated or calyculin A-treated (0.1 µM for 45 minutes prior to lysis) and subjected to 2-D gel electrophoresis, using Phospho-Threonine Antibody (P-Thr-Polyclonal).

Entrez-Gene ID #
Swiss-Prot Acc. #

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.

*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)	1:2000
IHC protocol: Unmasking buffer/Antibody diluent Citrate/TBST-5%NGS	
ELISA (Peptide)	1:1000

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

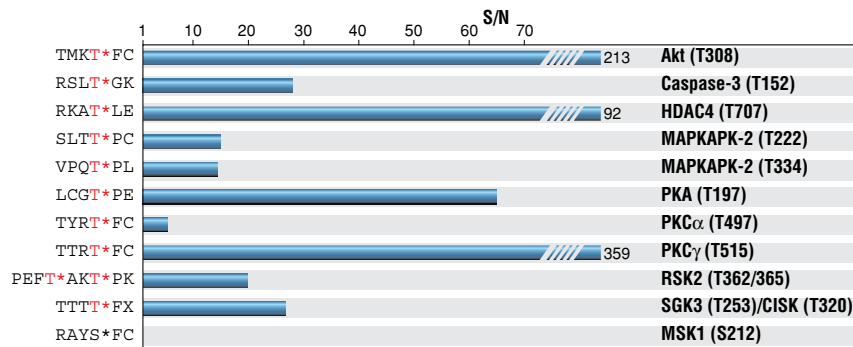
Companion Products:

- Phospho-Akt Substrate (RXRXXS/T) (110B7) Rabbit mAb #9614
- Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624
- Phospho-(Ser/Thr) PDK1 Docking Motif (18A2) Mouse mAb #9634
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

Please visit www.cellsignal.com for a complete listing of recommended companion products.

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 All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.



Phospho-Threonine Antibody (P-Thr-Polyclonal) DELFIA® Assay: Signal-to-noise ratio of phospho- versus nonphospho-peptides. (T* denotes phosphorylated threonine.) (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Selected Application References:

Haldrup, A. et al. (2000) Down-regulation of the PSI-F subunit of Photosystem I (PSI) in *Arabidopsis thaliana*. *Journal of Biological Chemistry* 275, 31211–31218. Application: W.

Ma, T. et al. (2000) Cell cycle-regulated phosphorylation of p220(NPAT) by cyclin E/Cdk2 in Cajal bodies promotes histone gene transcription. *Genes and Development* 14, 2298–2313. Application: W.

Scheinfeld, M.H. et al. (2003) Amyloid β protein precursor is phosphorylated by JNK-1 independent of, yet facilitated by, JNK-interacting protein (JIP)-1. *J. Biol. Chem.* 278, 42058–42063. Application: W.

Wada, S. et al. (2005) Phosphorylation of candida glabrata ATP-binding cassette transporter Cdr1p regulates drug efflux activity and ATPase stability. *J. Biol. Chem.* 280, 94–103. Application: W.

Gronborg, M. et al. (2002) A mass spectrometry-based proteomic approach for identification of serine/threonine-phosphorylated proteins by enrichment with phospho-specific antibodies: identification of a novel protein, Frigg, as a protein kinase A substrate. *Mol. Cell. Proteomics* 1, 517–527. Applications: IP, W.

Aoyama, K. et al. (2001) Molecular cloning and characterization of a novel dual specificity phosphatase, LMW-DSP2, that lacks the cdc25 homology domain. *J. Biol. Chem.* 276, 27575–27583. Application: W.

Background References:

- (1) Yaffe, M.B. and Elia, A.E. (2001) *Curr. Opin. Cell Biol.* 13, 131–138.
- (2) Appella, E. and Anderson, C.W. (2001) *J. Biochem. (Tokyo)* 268, 2764–2772.
- (3) Jenuwein, T. and Allis, C.D. (2001) *Science* 293, 1074–1080.
- (4) Krishna, R.G. and Wold, F. (1993) *Adv. Enzymol. Relat. Areas Mol. Biol.* 67, 265–298.
- (5) Venter, J.C. et al. (2000) *Science* 291, 1304–1351.
- (6) Manning, G. et al. (2002) *Science* 298, 1912–1934.