

# Phospho-(Thr) MAPK/CDK Substrate Mouse mAb

✓ 100 µl  
(50 western blots)

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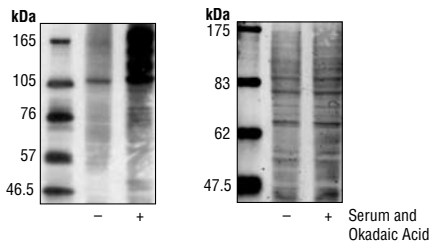
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Isotype	Motif
W, IHC-P, E-P	All	Mouse IgM**	T*P

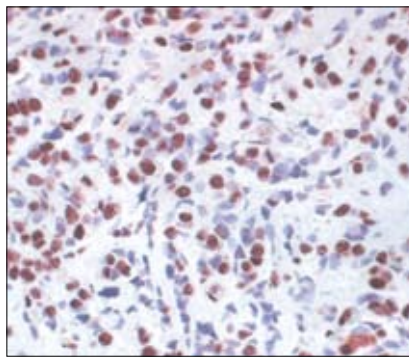
**Background:** The MAPK and CDK families of serine/threonine protein kinases play important roles in cell signaling and cell cycle control. These kinases phosphorylate threonine or serine followed by a proline residue (1-6). To study and discover new MAPK and CDK substrates, CST has developed antibodies that bind to phospho-threonine followed by proline.

As determined by ELISA using a wide variety of phospho-Thr-Pro peptides, Phospho-(Thr) MAPK/CDK Substrate Monoclonal Antibody recognizes the phospho-Thr-Pro motif in a highly context-independent fashion. It also interacts with a broad range of phospho-Thr-Pro-containing proteins as determined by Western blot analysis of nocodazole-treated Jurkat cell extracts resolved on a 2-D gel.

**Specificity/Sensitivity:** Phospho-(Thr) MAPK/CDK Substrate Mouse mAb detects phospho-threonine only when followed by proline. It reacts with proteins/peptides phosphorylated on the Thr-Pro motif in an otherwise highly context-independent fashion. The antibody does not cross-react with phospho-threonine in the absence of an adjacent proline. The antibody does not cross-react with phospho-tyrosine, but does react with some phospho-serine peptides containing the phospho-serine-proline motif (e.g., phospho-Elk-1). (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.)

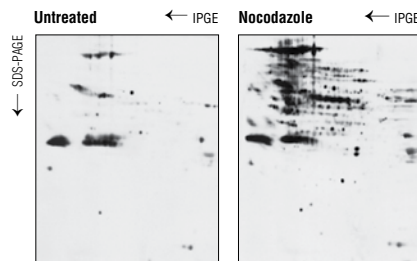


Western blot analysis of extracts from COS cells, untreated or treated with serum and okadaic acid, using Phospho-(Thr) MAPK/CDK Substrate Mouse mAb (left). The right panel shows total protein staining.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing staining of proteins containing phospho-threonine-proline motifs using Phospho-(Thr) MAPK/CDK Substrate Mouse mAb.

**Source/Purification:** Monoclonal antibody is produced by immunizing mice with synthetic phospho-threonine-proline-containing peptides (KLH-coupled). This antibody is a mouse IgM clone and can be recognized by anti-mouse Ig (whole molecule) secondary antibody. Antibody is purified by protein A chromatography.



Western blot analysis of extracts from Jurkat cells, untreated or nocodazole-treated (1 µg/ml for 12 hours prior to lysis) and subjected to 2-D gel electrophoresis using Phospho-(Thr) MAPK/CDK Substrate Mouse 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-mouse secondary antibodies must be used to detect this antibody.

**Recommended Antibody Dilutions:**

Western Blotting	1:5000
Immunohistochemistry (Paraffin)	1:50
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) Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol.* 200, 62-81.
- (2) Seger, R. and Krebs, E.G. (1995) *FASEB J.* 9, 726-735.
- (3) Nurse, P. (2000) *Cell* 100, 71-78.
- (4) Cross, T.G. et al. (2000) *Exp. Cell Res.* 256, 34-41.
- (5) Yang, C.C. et al. (1998) *J. Protein Chem.* 17, 329-335.
- (6) Reynolds, C.H. et al. (2000) *J. Neurochem.* 74, 1587-1595.

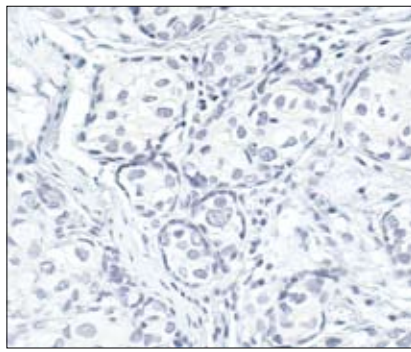
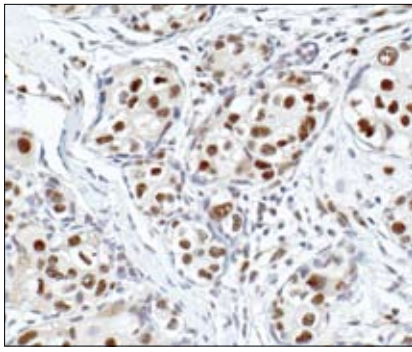
**IMPORTANT:** For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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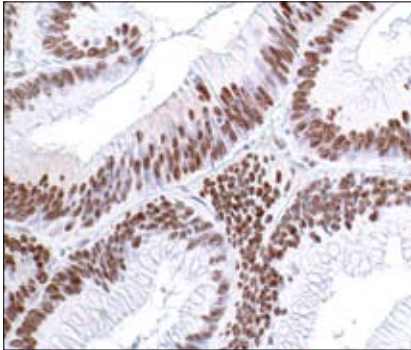
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA E-P—ELISA Peptide

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish B—bovine

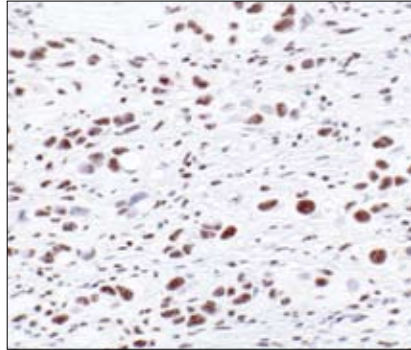
Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



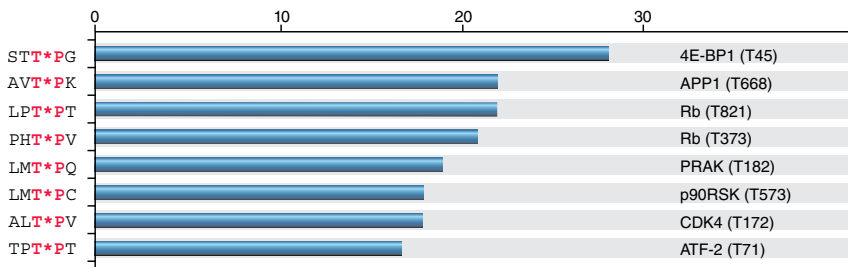
Immunohistochemical analysis of paraffin-embedded human breast carcinoma control (left) or  $\lambda$  phosphatase-treated (right), using Phospho-(Thr) MAPK/CDK Substrate Mouse mAb.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Phospho-(Thr) MAPK/CDK Substrate Mouse mAb.



Immunohistochemical analysis of paraffin-embedded human transitional epithelial carcinoma of the bladder using Phospho-(Thr) MAPK/CDK Substrate Mouse mAb.



Phospho-(Ser) CDKs Substrate Antibody ELISA Assay: Signal-to-noise ratio of phospho- versus nonphospho-peptides. (S\* denotes phosphorylated serine.)