#2351 Store at –20°C

Phospho-Threonine-X-Arginine Antibody

Cell Signaling TECHNOLOGY®

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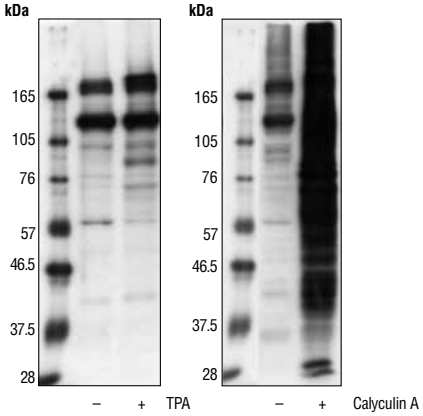
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Source	Motif
W, IHC-P, E-P	All	Rabbit**	T*X(K/R)

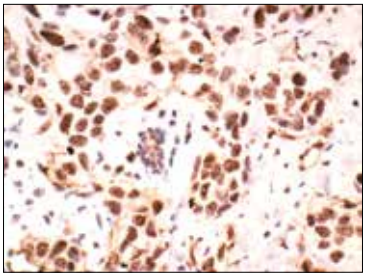
Background: Some signaling molecules can be regulated by phosphorylation at a specific threonine followed by arginine or lysine at the +2 position. For example, conventional PKC isozymes phosphorylate substrates containing serine or threonine with Arg or Lys at the -3, -2 and +2 positions (1–2). c-Raf, a mitogen-activated protein kinase and the main effector recruited by GTP-bound Ras, is phosphorylated at Thr481 and Thr491 followed by Lys at the +2 position (3). Phosphorylation of these sites is important for enzyme activities. To determine the phosphorylation state of Thr in the Thr-X-Arg motif, and to identify potential new phosphorylation sites with this motif, CST has developed a Phospho-Threonine X-Arginine Antibody that recognizes phosphorylated Thr followed by Arg or Lys at the +2 position.

Specificity/Sensitivity: Phospho-Threonine-X-Arginine Antibody detects endogenous levels of proteins containing the phospho-Thr-X-Arg motif. This antibody detects phosphorylated Thr followed by Arg or Lys at the +2 position, though its reactivity is lower for Lys compared to Arg at the +2 position. The antibody does not cross-react with nonphospho-Thr or phospho-Ser in the same motif. It recognizes phospho-Thr in the FFT*R motif in PKCβ II but does not recognize phospho-Thr in other motifs that lack Lys or Arg at +2. (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: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide containing the phospho-Thr-X-Arg motif. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from Jurkat cells, untreated, TPA-treated or calyculin A-treated, using Phospho-Threonine-X-Arginine Antibody.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing staining of proteins containing phosphorylated threonine-X-arginine motifs, using Phospho-Threonine-X-Arginine Antibody.

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
Immunohistochemistry (Paraffin)	1:800†
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.

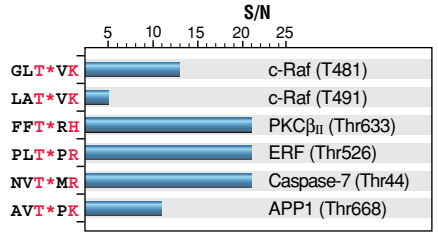
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Nishikawa, K. et al. (1997) *J. Biol. Chem.* 272, 952–960.

(2) Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol.* 200, 62–81.

(3) Zhang, B. and Guan, K. (2000) *EMBO J.* 19, 5429–5439.



Phospho-Threonine-X-Arginine Antibody ELISA: Signal-to-noise ratio of phospho- versus nonphospho-peptides containing the phospho-threonine-X-arginine motif. (T denotes phosphorylated threonine.)*

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.