Revision 1

#2351 Store at -20C

Phospho-Threonine-X-Arginine Antibody

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	Source:
WB, IHC-P, E-P	All	Endogenous	Rabbit

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunohistochemistry (Paraffin)	1:800
Peptide ELISA (DELFIA)	1:1000

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.

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 PKCbeta 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.

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, Cell Signaling Technology has developed a Phospho-Threonine X-Arginine Antibody that recognizes phosphorylated Thr followed by Arg or Lys at the +2 position.

Background References

1. Nishikawa, K. et al. (1997) *J Biol Chem* 272, 952-60.
2. Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol* 200, 62-81.
3. Zhang, B.H. and Guan, K.L. (2000) *EMBO J* 19, 5429-39.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

WB: Western Blotting **IHC-P:** Immunohistochemistry (Paraffin) **E-P:** Peptide ELISA (DELFIA)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **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 **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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Limited Uses

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