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Phospho-(Ser/Thr) ATM/ATR Substrate Antibody

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

Applications	Species Cross-Reactivity*	Source	Motif
W, IP, E-P Endogenous	All	Rabbit**	L(S*/T*)Q

Background: Ataxia telangiectasia mutated kinase (ATM) and ataxia telangiectasia and Rad3-related kinase (ATR) are related kinases that regulate cell cycle checkpoints and DNA repair (1). The identified substrates for ATM are p53, p95/NBS1, MDM2, Chk2, BRCA1, CtIP, 4E-BP1 and Chk1 (1,2) The essential requirement for the substrates of ATM/ATR is S*/T*Q. Hydrophobic amino acids at positions -3 and -1, and negatively charged amino acids at position +1 are positive determinants for substrate recognition by these kinases. Positively charged residues surrounding the S/TQ are negative determinants for substrate phosphorylation (3). The complex phenotype of AT cells suggests that it likely has additional substrates (3). To better understand the kinase and identify substrates for ATM and the related kinase ATR, CST has developed antibodies that recognize phosphorylated serine or threonine in the S*/T*Q motif. As shown by ELISA, Phospho-(Ser/Thr) ATM/ATR Substrate Antibody is specific for phosphorylated ATM/ATR substrates.

Specificity/Sensitivity: Phospho-(Ser/Thr) ATM/ATR Substrate Antibody detects endogenous levels of proteins containing the ATM/ATR substrate motif. This antibody preferentially binds peptides and proteins that contain phospho-Ser/Thr preceded by Leu or similar hydrophobic amino acids at the -1 position and followed by Gln at the +1 position. The antibody does not cross-react with corresponding nonphosphorylated sequences or with other phospho-Ser/Thr-containing motifs. (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.)

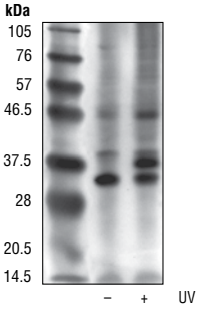
Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic phospho-(Ser/Thr) ATM/ATR substrate peptides (KLH-coupled). Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

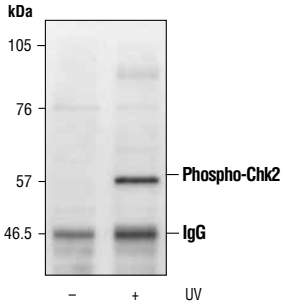
(1) Kastan, M.B. and Lim, D.S. (2000) *Nature Rev. Mol. Cell Biol.* 1, 179–186.

(2) Zhao, H. and Piwnica-Worms, H. (2001) *Mol. Cell. Biol.* 21, 4129–4139.

(3) Kim, S.T. et al. (1999) *J. Biol. Chem.* 274, 37538–37543.



Western blot analysis of extracts from COS cells, untreated or UV-treated, using Phospho-(Ser/Thr) ATM/ATR Substrate Antibody.



Western blot analysis of immunoprecipitated protein from Chk2-transfected and UV-treated COS cells, using Chk2 antibody for immunoprecipitation and Phospho-(Ser/Thr) ATM/ATR Substrate Antibody for immunoblotting.

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	1:20
ELISA-Peptide	1:1000

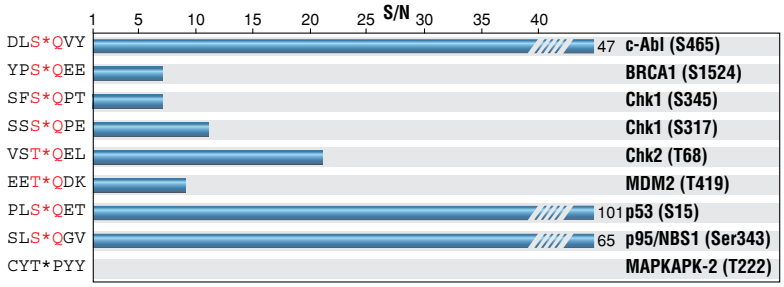
For application specific protocols please see the web page for this product at www.cellsignal.com.

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

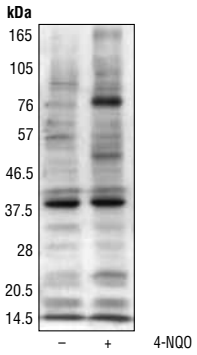
IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E**—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.



Phospho-(Ser/Thr) ATM/ATR Substrate Antibody ELISA Assay: Signal-to-noise ratio of phospho- versus nonphospho-peptides. (S or T* denote phosphorylated serine or threonine.)*



Western blot analysis of yeast cell extracts, untreated or treated with 4-NQO, a potent activator of ATM/ATR orthologues, using Phospho-(Ser/Thr) ATM/ATR Substrate Antibody.

Selected Application References:

Schwartz, M.F. et al. (2002) Rad9 phosphorylation sites couple Rad53 to the Saccharomyces cerevisiae DNA damage checkpoint. *Mol. Cell* 9, 1055–1065. Application: W.

DiTullio, R.A. et al. (2002) 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer. *Nat. Cell Biol.* 4, 998–1002. Application: IC-IF.

Ismail, I.H. et al. (2003) SU11752 inhibits the DNA-dependent protein kinase and DNA double-strand break repair resulting in ionizing radiation sensitization. *Oncogene*, Application: W.

Lou, Z. et al. (2003) Mediator of DNA damage checkpoint protein 1 regulates BRCA1 localization and phosphorylation in DNA damage checkpoint control. *J. Biol. Chem.* 278, 13599–13602. Application: IC-IF.

Lindstrom, M.S. and Wiman, K.G. (2003) Myc and E2F1 induce p53 through p14ARF-independent mechanisms in human fibroblasts. *Oncogene* 22, 4993–5005. Application: IC-IF.

Brumbaugh, K.M. et al. (2004) The mRNA surveillance protein hSMG-1 functions in genotoxic stress response pathways in mammalian cells. *Mol. Cell* 14, 585–598. Applications: IP, W.

d'Adda di Fagagna, F. et al. (2003) A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 426, 194–198. Applications: IC-IF, W.

Demonacos, C. et al. (2004) A new effector pathway links ATM kinase with the DNA damage response. *Nat. Cell Biol.* 6, 968–976. Application: W.

Uziel, T. et al. (2003) Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J.* 22 (20), 5612–5621. Application: IC-IF.

Lee, S. et al. (2003) Rad53 phosphorylation site clusters are important for Rad53 regulation and signaling. *Mol. Cell. Biol.* 23 (17), 6300–6314. Application: W.