

Phospho-Chk2 (Ser516) Antibody



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Entrez-Gene ID #11200
UniProt ID #O96017

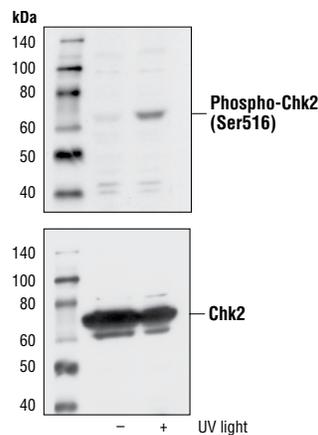
Applications W Endogenous	Species Cross-Reactivity* H	Molecular Wt. 62 kDa	Source Rabbit**
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Background: Chk2 is the mammalian orthologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (1–3). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50 and Thr68) each followed by glutamine (SQ or TQ motif). These are known to be preferred sites for phosphorylation by ATM/ATR kinases (4,5). After DNA damage by ionizing radiation (IR), UV irradiation or hydroxyurea treatment, Thr68 and other sites in this region become phosphorylated by ATM/ATR (5–7). The SQ/TQ cluster domain, therefore, seems to have a regulatory function. Phosphorylation at Thr68 is a prerequisite for the subsequent activation step, which is attributable to auto-phosphorylation of Chk2 on residues Thr383 and Thr387 in the activation loop of the kinase domain (8).

Chk2 autophosphorylation at Ser516 is important for optimal Chk2 function, and a Ser516Ala mutant Chk2 is defective in IR-induced apoptosis (9).

Specificity/Sensitivity: Phospho-Chk2 (Ser516) Antibody detects endogenous levels of Chk2 only when phosphorylated at serine 516. The antibody does not cross-react with Chk2 phosphorylated at other sites.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser516 of human Chk2. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from 293 cells, untreated or UV-treated (50 mJ/cm², 2 hrs), using Phospho-Chk2 (Ser516) Antibody (upper) or Chk2 Antibody #2662 (lower).

Background References:

- (1) Allen, J.B. et al. (1994) *Genes Dev.* 8, 2401–2415.
- (2) Weinert, T.A. et al. (1994) *Genes Dev.* 8, 652–665.
- (3) Murakami, H. and Okayama, H. (1995) *Nature* 374, 817–819.
- (4) Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179–186.
- (5) Matsuoka, S. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 10389–10394.
- (6) Melchionna, R. et al. (2000) *Nat. Cell Biol.* 2, 762–765.
- (7) Ahn, J.Y. et al. (2000) *Cancer Res.* 60, 5934–5936.
- (8) Lee, C.H. and Chung, J.H. (2001) *J. Biol. Chem.* 276, 30537–30541.
- (9) Wu, X. and Chen, J. (2003) *J. Biol. Chem.* 278, 36163–36168.

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

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

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.

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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.