#2665 Store at –20°C

Phospho-Chk2 (Ser33/35) Antibody

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

Entrez-Gene ID #11200
UniProt ID #O96017

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, Mk	62 kDa	Rabbit**

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).

Specificity/Sensitivity: Phospho-Chk2 (Ser33/35) Antibody detects endogenous levels of Chk2 only when phosphorylated at serine 33/35. 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 Ser33/35 of human Chk2. Antibodies are purified by protein A and peptide affinity chromatography.

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.

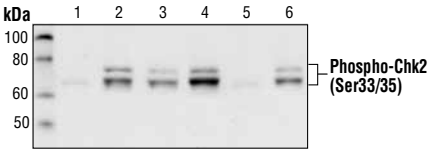
(4) Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179–186.

(5) Matsuoka, S. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 10389–10394.

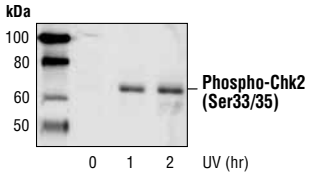
(6) Melchionna, R. et al. (2000) *Nat. Cell Biol.* 2, 762–765.

(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.



Western blot analysis of extracts from COS cells, untransfected (lane 1) or transfected with Wild-type Chk2 (lane 2), Chk2 (S19A) (lane 3), Chk2 (T26S28A) (lane 4), Chk2 (S33S35A) (lane 5) or Chk2 (T68A) (lane 6), using Phospho-Chk2 (Ser33/35) Antibody.



Western blot analysis of extracts from HeLa cells treated with UV for the indicated times, using Phospho-Chk2 (Ser33/35) 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

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.

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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**—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.