#12302 Store at -20°C

Phospho-Chk1 (Ser317) (D12H3) XP® Rabbit mAb

☐ Small 100 µl
(10 western blots)

☐ Petite 40 µl
(4 western blots)

Cell Signaling TECHNOLOGY®

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

Entrez-Gene ID #1111
UniProt Acc. #O14757

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC	H, M, Mk	56 kDa	Rabbit IgG**
Endogenous			

Background: Chk1 kinase acts downstream of ATM/ATR kinase and plays an important role in DNA damage checkpoint control, embryonic development, and tumor suppression (1). Activation of Chk1 involves phosphorylation at Ser317 and Ser345 and occurs in response to blocked DNA replication and certain forms of genotoxic stress (2). While phosphorylation at Ser345 serves to localize Chk1 to the nucleus following checkpoint activation (3), phosphorylation at Ser317 along with site-specific phosphorylation of PTEN allows for re-entry into the cell cycle following stalled DNA replication (4). Chk1 exerts its checkpoint mechanism on the cell cycle, in part, by regulating the cdc25 family of phosphatases. Chk1 phosphorylation of cdc25A targets it for proteolysis and inhibits its activity through 14-3-3 binding (5). Activated Chk1 can inactivate cdc25C via phosphorylation at Ser216, blocking the activation of cdc2 and transition into mitosis (6). Centrosomal Chk1 has been shown to phosphorylate cdc25B and inhibit its activation of CDK1-cyclin B1, thereby abrogating mitotic spindle formation and chromatin condensation (7). Furthermore, Chk1 plays a role in spindle checkpoint function through regulation of aurora B and BubR1 (8). Research studies have implicated Chk1 as a drug target for cancer therapy as its inhibition leads to cell death in many cancer cell lines (9).

Specificity/Sensitivity: Phospho-Chk1 (Ser317) (D12H3) XP® Rabbit mAb recognizes endogenous levels of Chk1 protein only when phosphorylated at Ser317. This antibody also detects an 80 kDa protein of unknown origin in some cell lines.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser317 of human Chk1 protein.

Background References:

(1) Liu, Q. et al. (2000) *Genes Dev* 14, 1448-59.
(2) Zhao, H. and Piwnica-Worms, H. (2001) *Mol Cell Biol* 21, 4129-39.
(3) Jiang, K. et al. (2003) *J Biol Chem* 278, 25207-17.
(4) Martin, S.A. and Ouchi, T. (2008) *Mol Cancer Ther* 7, 2509-16.
(5) Chen, M.S. et al. (2003) *Mol Cell Biol* 23, 7488-97.
(6) Zeng, Y. et al. (1998) *Nature* 395, 507-10.
(7) Löffler, H. et al. (2006) *Cell Cycle* 5, 2543-7.
(8) Zachos, G. et al. (2007) *Dev Cell* 12, 247-60.
(9) Garber, K. (2005) *J Natl Cancer Inst* 97, 1026-8.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

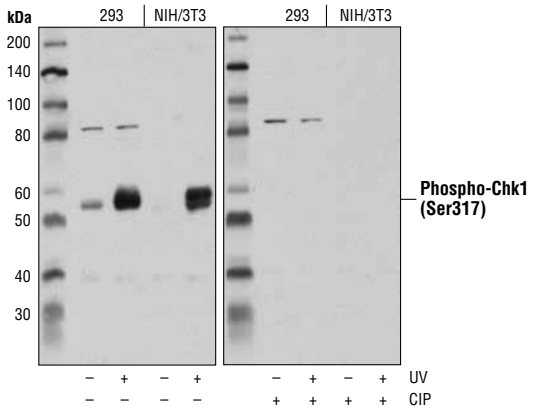
****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

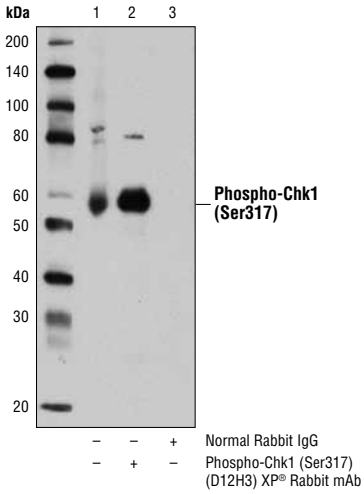
Western blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (IF-IC)	1:800

For product 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.



Western blot analysis of extracts from 293 and NIH-3T3 cells, untreated (-) or UV-treated (100 mJ, 1 hr recovery; +), using Phospho-Chk1 (Ser317) (D12H3) XP® Rabbit mAb. The blot on the right was treated with calf intestinal phosphatase (CIP) before western blot.

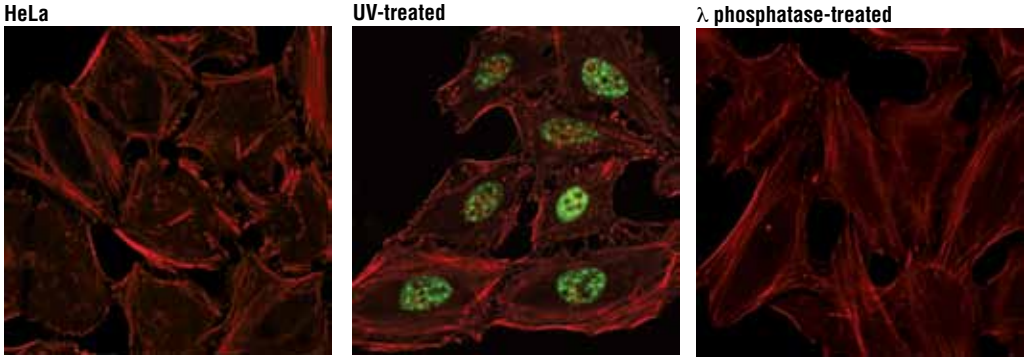


Immunoprecipitation of phospho-Chk1 (Ser317) from 293 cell extracts treated with UV (100 mJ, 1 hr recovery) using Phospho-Chk1 (Ser317) (D12H3) XP® Rabbit mAb (lane 2) or Rabbit (D1AG) mAb IgG XP® Isotype Control #3900 (lane 3). Lane 1 is 10% input.

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.



Confocal immunofluorescent analysis of HeLa cells, untreated (left), UV-treated (center), or UV and λ phosphatase-treated (right), using Phospho-Chk1 (Ser317) (D12H3) XP® Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).