Store at –20°C

#2197

Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb

☐ Small 100 µl
(10 western blots)

☐ Petite 40 µl
(4 western blots)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
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rev. 03/24/10

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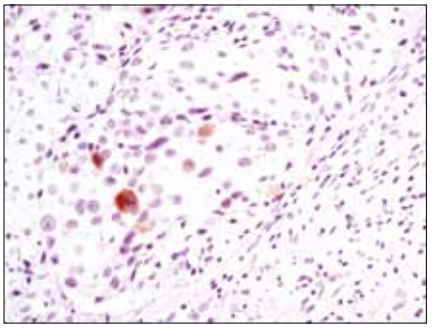
Entrez-Gene ID #11200
Swiss-Prot Acc. #O96017

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, F Endogenous	H, (Mk)	62 kDa	Rabbit IgG**

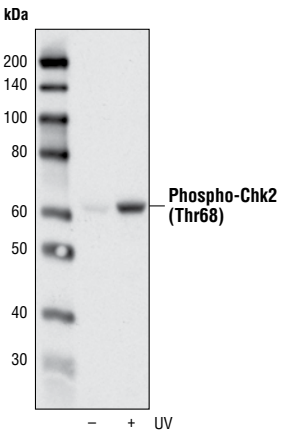
Background: Chk2 is the mammalian orthologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (1-3). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50 and Thr68) each followed by glutamine (SQ or TQ 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 (Thr68) (C13C1) Rabbit mAb detects endogenous levels of Chk2 only when phosphorylated at Thr68.

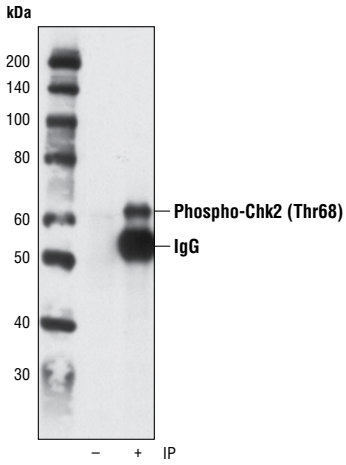
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr68 of human Chk2.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb.



Western blot analysis of extracts from HeLa cells, untreated or UV-treated, using Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb.



Immunoprecipitation of phospho-chk2 from UV-treated HT29 cells using Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb followed by western blot using the same antibody.

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.

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	1:100
Immunohistochemistry (Paraffin)	1:200
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Flow Cytometry	1:50

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

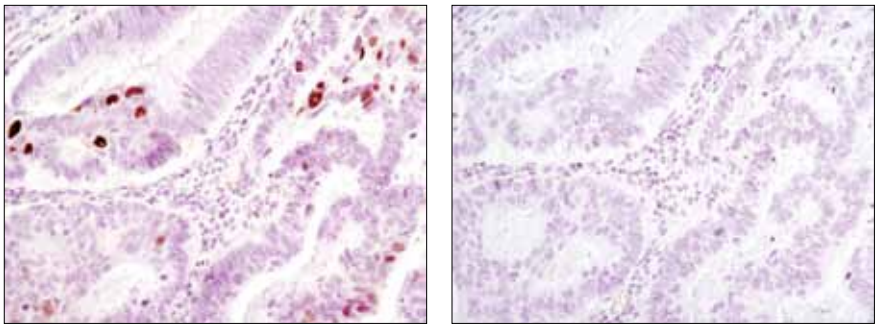
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

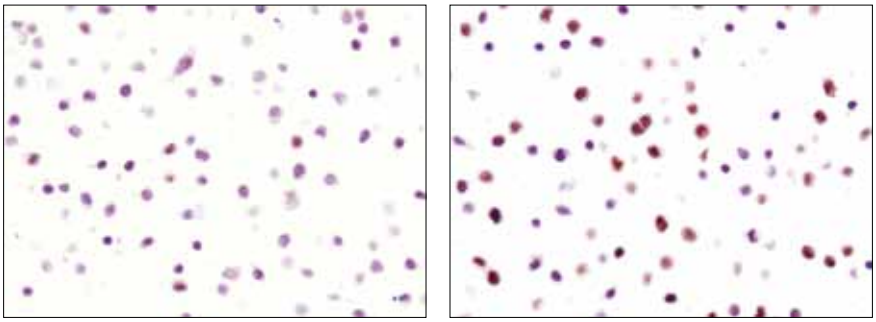
(1) Allen, J.B. et al. (1994) *Genes Dev.* 8, 2401–2415.
(2) Weinert, T.A. et al. (1994) *Genes Dev.* 8, 652–665.
(3) Murakami, H. and Okayama, H. (1995) *Nature* 374, 817–819.
(4) Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179–186.
(5) Matsuoka, S. et al. (2000) *Proc. Natl. Acad. 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.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

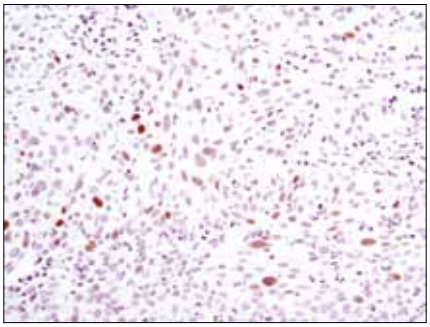
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



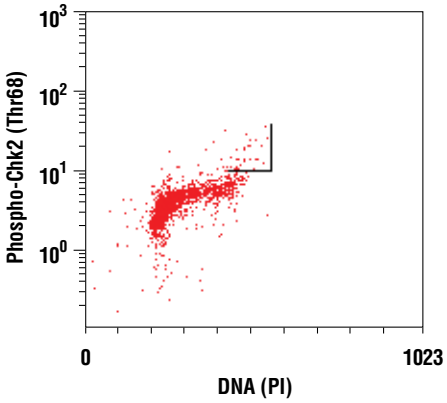
Immunohistochemical analysis of paraffin-embedded human colon carcinoma, control (left) or λ phosphatase-treated (right), using Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded HT-29 cell pellets, control (left) or UV-treated (right), using Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb.



Flow cytometric analysis of untreated Jurkat cells using Phospho-Chk2 (Thr68) (C13C1) Rb mAb versus propidium iodide (DNA content). The boxed population indicates phospho-Chk2 (Thr68)-positive cells.

Chk2 (D9C6) XP® Rabbit mAb

#6334 Store at -20°C

☐ Small 100 µl (10 western blots)
☐ Petite 40 µl (4 western blots)

New 05/12

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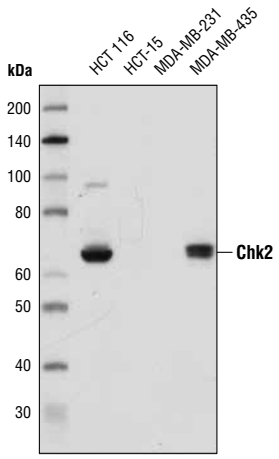
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC	H	62 kDa	Rabbit IgG**
Endogenous			

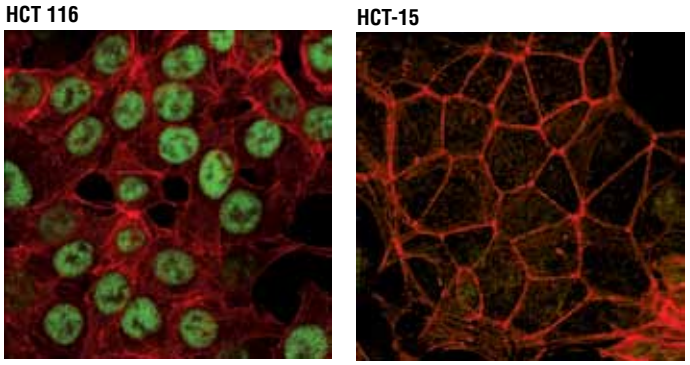
Background: Chk2 is the mammalian orthologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (1-3). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50, and Thr68) each followed by glutamine (SQ or TQ 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 at residues Thr383 and Thr387 in the activation loop of the kinase domain (8).

Specificity/Sensitivity: Chk2 (D9C6) XP® Rabbit mAb recognizes endogenous levels of total Chk2 protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a recombinant protein specific to human Chk2 protein.



Western blot analysis of extracts from various cell lines using Chk2 (D9C6) XP® Rabbit mAb.



Confocal immunofluorescent analysis of HCT 116 (left) and HCT-15 (right) cells using Chk2 (D9C6) XP® Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red).

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.

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	1:100
Immunohistochemistry (Paraffin)	1:400†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:200

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

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(1) Allen, J.B. et al. (1994) *Genes Dev.* 8, 2401-2415.
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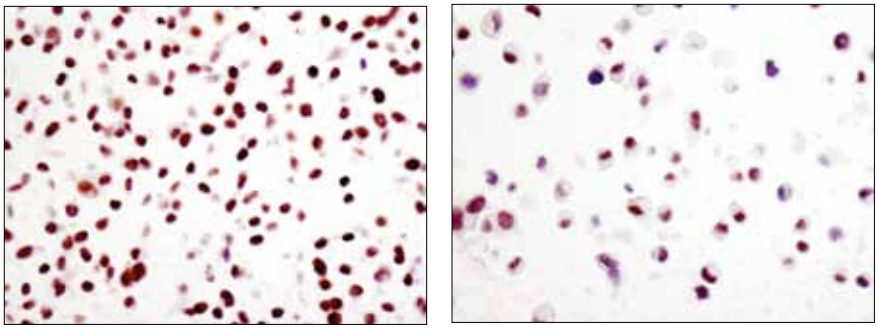
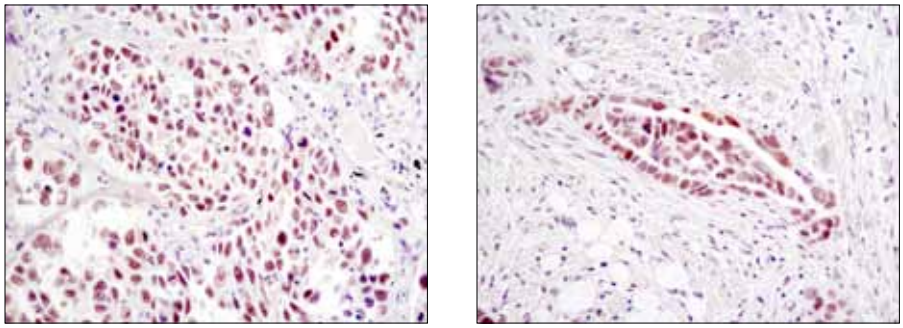
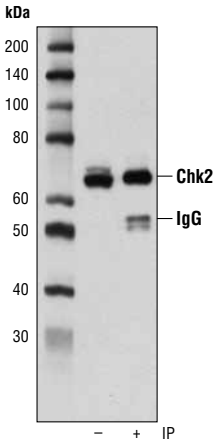


Immunohistochemical analysis of paraffin-embedded cell pellets, HCT 116 (left) or MDA-MB-231 (right), using Chk2 (D9C6) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcioma using Chk2 (D9C6) XP® Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded human ovarian serous adenocarcinoma using Chk2 (D9C6) XP® Rabbit mAb.



Immunoprecipitation of Chk2 from 293 cell extracts using Chk2 (D9C6) XP® Rabbit mAb (lane 2). Western blot detection was performed using the same antibody. Lane 1 is 10% input.