#9931 Store at -20°C

Phospho-Chk1/2 Antibody Sampler Kit

✓ 1 Kit
(9 x 40 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
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rev. 11/16/12

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Chk1 (Ser317) Antibody	2344	40 µl	56 kDa	Rabbit IgG
Phospho-Chk1 (Ser345) (133D3) Rabbit mAb	2348	40 µl	56 kDa	Rabbit IgG
Phospho-Chk2 (Ser19) Antibody	2666	40 µl	62 kDa	Rabbit IgG
Phospho-Chk2 (Ser33/35) Antibody	2665	40 µl	62 kDa	Rabbit IgG
Phospho-Chk2 (Thr68) Antibody	2661	40 µl	62 kDa	Rabbit IgG
Phospho-Chk2 (Ser516) Antibody	2669	40 µl	62 kDa	Rabbit IgG
Phospho-Chk1 (Ser296) Antibody	2349	40 µl	56 kDa	Rabbit IgG
Chk1 (2G1D5) Mouse mAb	2360	40 µl	56 kDa	Mouse IgG1
Chk2 Antibody	2662	40 µl	62 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

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

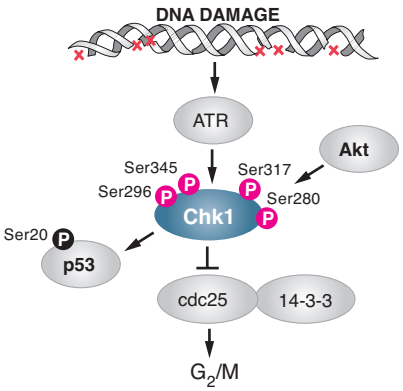
Description: The Phospho-Chk1/2 Antibody Sampler Kit offers an economical means to evaluate the phosphorylation status of Chk1 and Chk2 on multiple residues. The kit contains enough primary and secondary antibodies to perform four Western blot experiments with each primary antibody.

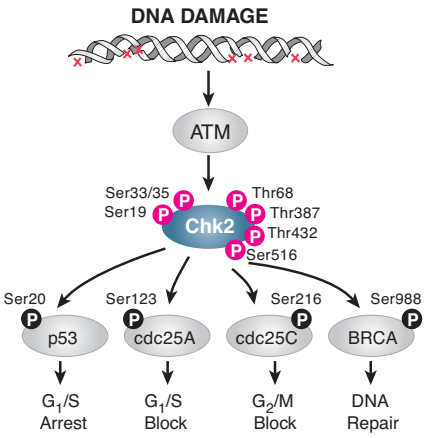
Background: Chk1 kinase acts downstream of ATM/ATR kinase and plays an important role in DNA damage checkpoint control, embryonic development, and tumor suppression (1). Activation of Chk1 involves phosphorylation at Ser317 and Ser345 and occurs in response to blocked DNA replication and certain forms 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 reentry 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).

Chk2 is the mammalian homologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (5-7). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50 and Thr68) followed by glutamine (SQ or TQ motif). These are known to be preferred sites for phosphorylation by ATM/ATR kinases (8). Indeed, after DNA damage by ionizing radiation (IR), UV irradiation and DNA replication blocked by hydroxyurea, Thr68 and other sites in this region become phosphorylated by ATM/ATR (9-11). 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 autophosphorylation of Chk2 on residues Thr383 and Thr387 in the activation loop of the kinase domain (12).

Specificity/Sensitivity: Each phospho-specific Chk1 antibody detects endogenous levels of Chk1 when phosphorylated at the indicated site (Ser296, Ser317 or Ser345), and each phospho-specific Chk2 antibody detects endogenous levels Chk2 only when phosphorylated at the indicated site (Ser19, Ser33/35, Thr68, and Ser516). Chk1 (2G1D5) Mouse mAb #2360 detects endogenous levels of total Chk1. Chk2 Antibody #2662 detects endogenous levels of total Chk2.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser345 of human Chk1. Polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser296 or Ser317 of human Chk1 and Ser19, Ser33/35, Thr68, Ser516 of human Chk2. Chk1 (2G1D5) Mouse mAb #2360 is produced by immunizing animals with purified recombinant Chk1 protein. Chk 2 Antibody #2662 is produced by immunizing rabbits with a synthetic peptide corresponding to amino-terminal residues of human Chk2. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.





Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.

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.

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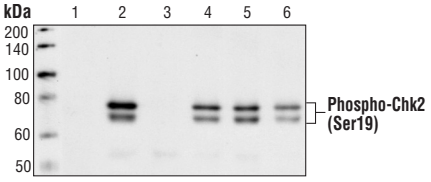
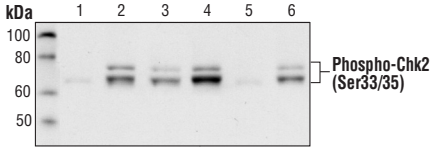
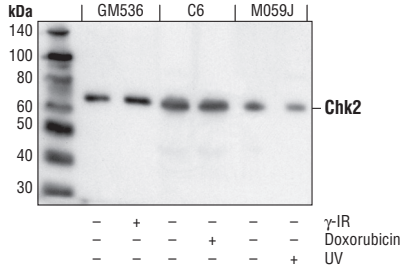


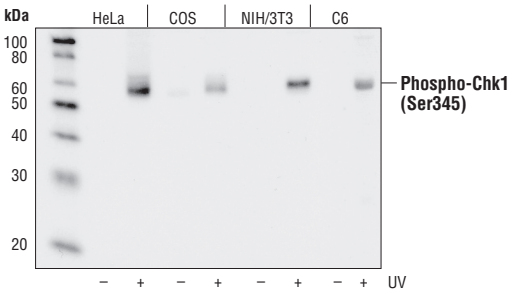
Western blot analysis of extracts from COS cells, untransfected (lane 1) or transfected with Wild-type Chk2 (lane 2), Chk2 (S19A) (lane 3), Chk2 (T26S28A) (lane 4), Chk2 (S33S35A) (lane 5) or Chk2 (T68A) (lane 6), using ***Phospho-Chk2 (Ser19) Antibody #2666.***



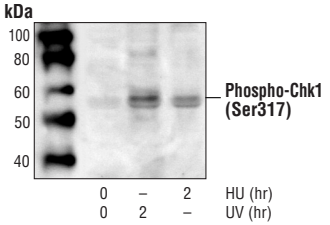
Western blot analysis of extracts from COS cells, untransfected (lane 1) or transfected with Wild-type Chk2 (lane 2), Chk2 (S19A) (lane 3), Chk2 (T26S28A) (lane 4), Chk2 (S33S35A) (lane 5) or Chk2 (T68A) (lane 6), using ***Phospho-Chk2 (Ser33/35) Antibody #2665.***



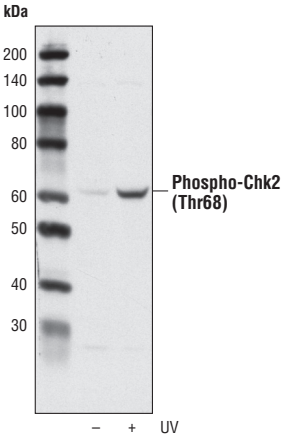
Western blot analysis of extracts from GM536, C6, and M059J cells using ***Chk2 Antibody #2662.***



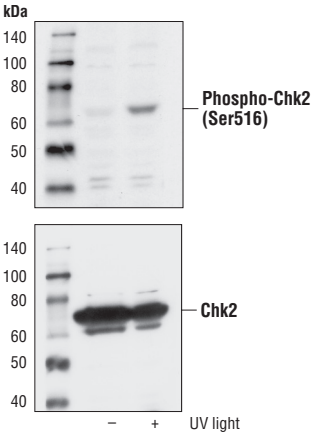
Western blot analysis of extracts from HeLa, COS, NIH/3T3 and C6 cells, untreated or UV-treated, using ***Phospho-Chk1 (Ser345) (133D30) Rabbit mAb #2348***.



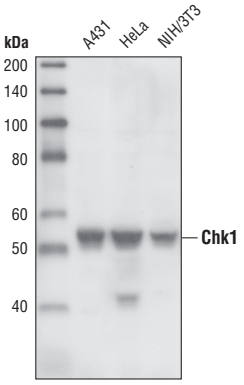
Western blot analysis of extracts from MvlLu cells treated with UV or HU as indicated, using ***Phospho-Chk1 (Ser317) Antibody #2344.***



Western blot analysis of extracts from Cos cells, untreated or UV-treated (100 mJ/cm², 1 hr recovery), using ***Phospho-Chk2 (Thr68) Antibody #2661.***



Western blot analysis of extracts from 293 cells, untreated or UV-treated (50 mJ/cm2, 2hrs), using ***Phospho-Chk2 (Ser516) Antibody #2669*** *(upper) or Chk2 Antibody #2662 (lower).*



Western blot analysis of extracts from various cell lines using ***Chk1 (2G1D5) Mouse mAb #2360***.

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.

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

(11) Weinert, T. A. et al. (1994) *Genes Dev.* 8, 652-665.

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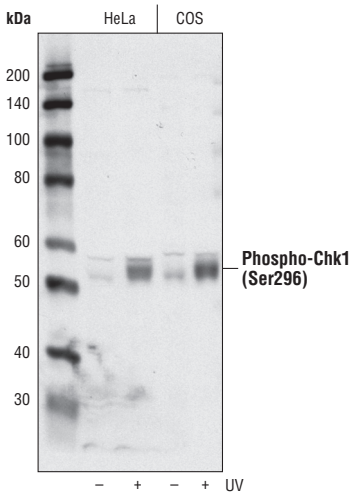
(13) Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179-186.

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

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

(16) Ahn, J. Y. et al. (2000) *Cancer Res.* 60, 5934-5936.



Western blot analysis of extracts from Hela and Cos cells, untreated or treated with 100 mJ/cm2 UV light with 1 hour recovery, using ***Phospho-Chk1 (Ser296) Antibody, #2349.***

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Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.

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