Store at -20°C

#9917

Cell Cycle/Checkpoint Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

Orders ■ 877-616-CELL (2355)
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Support ■ 877-678-TECH (8324)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-cdc2 (Tyr15) (10A11) Rabbit mAb	4539	20 µl	34 kDa	Rabbit IgG
Phospho-Rb (Ser807/811) (D20B12) XP® Rabbit mAb	8516	20 µl	110 kDa	Rabbit IgG
Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb	2197	20 µl	62 kDa	Rabbit IgG
Phospho-Rb (Ser795) Antibody	9301	20 µl	110 kDa	Rabbit IgG
Phospho-Chk1 (Ser345) (133D3) Rabbit mAb	2348	20 µl	56 kDa	Rabbit IgG
Phospho-p53 (Ser15) (16G8) Mouse mAb	9286	20 µl	53 kDa	Mouse IgG1
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse
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 validation data and a complete listing of recommended companion products.

Description: The Cell Cycle/Checkpoint Antibody Sampler Kit provides an economical means of evaluating multiple proteins involved in the cell cyle and checkpoint control. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The cell division cycle demands accuracy to avoid the accumulation of genetic damage. This process is controlled by molecular circuits called "checkpoints" that are common to all eukaryotic cells (1). Checkpoints monitor DNA integrity and cell growth prior to replication and division at the G1/S and G2/M transitions, respectively. The cdc2-cyclin B kinase is pivotal in regulating the G2/M transition (2,3). Cdc2 is phosphorylated at Thr14 and Tyr15 during G2-phase by the kinases Wee1 and Myt1, rendering it inactive. The tumor suppressor protein retinoblastoma (Rb) controls progression through the late G1 restriction point (R) and is a major regulator of the G1/S transition (4). During early and mid G1-phase, Rb binds to and represses the transcription factor E2F (5). The phosphorylation of Rb late in G1-phase by CDKs induces Rb to dissociate from E2F, permitting the transcription of S-phase-promoting genes. *In vitro*, Rb can be phosphorylated at multiple sites by cdc2, cdk2, and cdk4/6 (6-8). DNA damage triggers both the G2/M and the G1/S checkpoints. DNA damage activates the DNA-PK/ATM/ATR kinases, which phosphorylate Chk at Ser345 (9), Chk2 at Thr68 (10) and p53 (11). The Chk kinases inactivate cdc25 via phosphorylation at Ser216, blocking the activation of cdc2.

Specificity/Sensitivity: Phospho-cdc2 (Tyr15) (10A11) Rabbit mAb detects endogenous levels of cdc2 protein only when phosphorylated at tyrosine 15. Based on sequence similarity, the antibody may cross-react with CDK2 and CDK3. Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb detects endogenous levels of Chk2 only when phosphorylated at Thr68. Phospho-Chk1 (Ser345) Antibody detects Chk1 only when phosphorylated at Ser345 and does not cross-react with other proteins. Phospho-Rb (Ser795) Antibody detects Rb only when phosphorylated at Ser795 and does not cross-react with Rb phosphorylated at other sites. Phospho-Rb (Ser807/811) (D20B12) XP® Rabbit mAb recognizes endogenous levels of Rb protein only when phosphorylated at Ser807, Ser811, or at both sites. This antibody does not cross-react with Rb phosphorylated at Ser608. Phospho-p53 (Ser15) (16G8) Mouse mAb detects endogenous levels of p53 only when phosphorylated at Ser15. The antibody does not cross-react with p53 phosphorylated at other sites.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser795 of human Rb. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser807/811 of human Rb protein, residues surrounding Ser345 of human Chk1, residues surrounding Ser15 of human p53, residues surrounding Tyr15 of human cdc2, and residues surrounding Thr68 of human Chk2.

Background References:

(1) Nurse, P. (1997) *Cell* 91, 865–7.
(2) Norbury, C. and Nurse, P. (1992) *Annu Rev Biochem* 61, 441–70.
(3) Watanabe, N. et al. (1995) *EMBO J.* 14, 1878–1891.
(4) Sherr, C.J. (1996) *Science* 274, 1672–1677.
(5) Dyson, N. (1998) *Genes Dev.* 12, 2245–2262.
(6) Kitagawa, M. et al. (1996) *EMBO J.* 15, 7060–7069.
(7) Lundberg, A.S. and Weinberg, R.A. (1998) *Mol Cell Biol* 18, 753–761.
(8) Harbour, J.W. et al. (1999) *Cell* 98, 859–869.
(9) Zhao, H. and Piwnica-Worms, H. (2001) *Mol. Cell Biol.* 21, 4129–4139.
(10) Matsuoka, S. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 10389–10394.
(11) Tibbetts, R.S. et al. (1999) *Genes Dev.* 13, 152–157.

U.S. Patent No. 5,675,063

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 Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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