Store at –20°C

#9870

Cell Cycle Regulation Antibody Sampler Kit II

✔1 Kit
(8 x 20 µl)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/16

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
Cyclin A (BF683) Mouse mAb	4656	20 µl	55 kDa	Mouse IgE
Cyclin B1 (D5C10) XP® Rabbit mAb	12231	20 µl	55 kDa	Rabbit IgG
Cyclin E2 Antibody	4132	20 µl	48 kDa	Rabbit IgG
Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb	3377	20 µl	17 kDa	Rabbit IgG
Myt1 Antibody	4282	20 µl	60-70 kDa	Rabbit IgG
p21 Waf1/Cip1 (12D1) Rabbit mAb	2947	20 µl	21 kDa	Rabbit IgG
Phospho-Wee1 (Ser642) (D47G5) Rabbit mAb	4910	20 µl	95 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

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 antibodies.*

Recommended Antibody Dilutions:

Western blotting 1:1000

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

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

Description: The Cell Cycle Regulation Sampler Kit II provides an economical means of evaluating cell cycle proteins. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The critical regulatory step in activating cdc2 during progression into mitosis appears to be dephosphorylation of cdc2 at Tyr15 and Thr14 (1). Phosphorylation of cdc2 by the protein kinases Wee1 and Myt1 at Thr14 and Tyr15 results in inhibition of cdc2 (2,3). Progression through the G1/S checkpoint and initiation of DNA replication requires cyclin E; traversing the G2/M checkpoint to initiate mitosis requires cyclin B, and cyclin A may be required for both S-phase and M-phase (4). The versatile p21 cyclin-dependent kinase inhibitor, which interacts with several cyclin-CDK complexes to regulate cyclin-CDK during the cell cycle, is regulated by phosphorylation and ubiquitin-mediated degradation (5). Phosphorylation of histone H3 at Ser10 and neighboring residues correlates with chromosomal condensation, which is essential for segregation of chromosomes during mitosis (6).

Specificity/Sensitivity: Each antibody in the Cell Cycle Regulation Sampler Kit II detects endogenous levels of its target protein and does not typically cross react with other family members. Cyclin B1 (D5C10) XP® Rabbit mAb recognizes endogenous levels of total cyclin B1 protein. This antibody also detects a 100 kDa protein of unknown origin in some cell lines. Activation state antibodies recognize target proteins only when phosphorylated at the indicated residue. 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.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues near the carboxy-terminus of human cyclin E2 or the middle of mouse and human Myt1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to residues near the carboxy-terminus of human p21, residues near the amino terminus of human cyclin B1 protein, or recombinant human cyclin A2 protein. Activation state monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser10 of human histone H3, residues surrounding Ser642 of human Wee1, or residues surrounding Tyr15 of human cdc2.

Background References:

(1) Norbury, C. et al. (1991) *EMBO J* 10, 3321-9.

(2) McGowan, C.H. and Russell, P. (1993) *EMBO J* 12, 75-85.

(3) Wells, N.J. et al. (1999) *J Cell Sci* 112 (Pt 19), 3361-71.

(4) Pagano, M. et al. (1992) *EMBO J* 11, 961-71.

(5) Abbas, T. and Dutta, A. (2009) *Nat Rev Cancer* 9, 400-14.

(6) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-60.

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.

© 2014 Cell Signaling Technology, Inc.
XP and Cell Signaling Technology are trademarks of Cell Signaling Technology, Inc.

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.

LumiGLO® is a registered trademark of Kirkegaard & Perry Laboratories. Tween® is a registered trademark of ICI Americas, INC. SignalFire™ is a trademark of Cell Signaling Technology, INC.

© 2014 Cell Signaling Technology, Inc.