#9110 Store at -20°C

PhosphoPlus® cdc2 (Tyr15) Antibody Kit

☑ 1 Kit
(10 western blots)

Cell Signaling TECHNOLOGY®

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rev. 03/31/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-cdc2 (Tyr1556) Antibody	9111	100 µl	34 kDa	Rabbit IgG
cdc2 (POH1) Mouse mAb	9116	100 µl	34 kDa	Mouse IgG2a
cdc2 (Tyr15) Control Proteins	9113	80 µl	32, 34 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse
Anti-biotin, HRP-linked Antibody	7075	100 µl		Goat
Biotinylated Protein Ladder Detection Pack	7727	100 µl		
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml		

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, 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

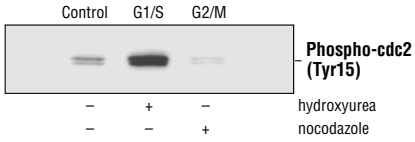
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Description: PhosphoPlus® Cdc2 (Tyr15) Western Detection Kit offers an efficient way of detecting cdc2 (Tyr15) phosphorylation by western blotting. The kit contains enough primary and secondary antibodies to perform 10 western blots, as well as a set of pre-stained and biotinylated markers, control proteins and LumiGLO® reagent.

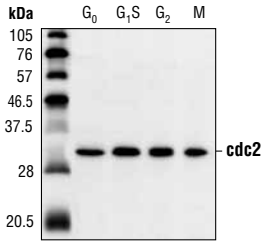
Background: The entry of eukaryotic cells into mitosis is regulated by cdc2 kinase activation, a process controlled at several steps including cyclin binding and phosphorylation of cdc2 at Thr161 (1). However, the critical regulatory step in activating cdc2 during progression into mitosis appears to be dephosphorylation of cdc2 at Thr14 and Tyr15 (2). Phosphorylation at Thr14 and Tyr15, resulting in inhibition of cdc2, can be carried out by Wee1 and Myt1 protein kinases (3,4). The cdc25 phosphatase may be responsible for removal of phosphates at Thr14 and Tyr15 and subsequent activation of cdc2 (1,5).

Specificity/Sensitivity: When used in conjunction with our Phototope®-HRP Western Detection Kit, Phospho-cdc2 (Tyr15) Antibody detects less than 10 ng of tyrosine phosphorylated cdc2 yet will not react with up to 1 µg of non-tyrosine phosphorylated cdc2. Similarly, Phospho-cdc2 (Tyr15) Antibody detects phosphorylated tyrosine 15 of cdk2 but demonstrates no cross reactivity to cdk4, cdk6 and cdk7 or other phospho-tyrosine proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the phsphorylation site. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a recombinant human cdc2 fusion protein.



Western blot analysis of extracts from Saos cells, either untreated or treated with hydroxyurea or nocodazole, using ***Phospho-cdc2 (Tyr15) Antibody #9111****.*



Western blot analysis of extracts from HeLa cells synchronized at various stages of the cell cycle, using ***cdc2 (POH1) Mouse mAb #9116****.*

Background References:

(1) Atherton-Fessler, S. et al. (1994) *Mol. Biol. Cell.* 5, 989-1001.

(2) Norbury, C. et al. (1991) *EMBO. J.* 10, 3321-3329.

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

(4) Wells, N.J. et al. (1999) *J. Cell. Sci.* 112, 3361-3371.

(5) Hunter, T. (1995) *Cell* 80, 225-236.

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.

#9110

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.