

#9110 Store at -20°C

# PhosphoPlus® cdc2 (Tyr15) Antibody Kit



✓ 1 Kit  
(10 western blots)

**Orders** ■ 877-616-CELL (2355)  
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-cdc2 (Tyr1556) Antibody	9111	100 µl	34 kDa	Rabbit IgG
cdc2 Antibody	9112	100 µl	34 kDa	Rabbit IgG
cdc2 (Tyr15) Control Proteins	9113	100 µl	32, 34 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-biotin, HRP-linked (D5A7) Rabbit mAb	5571	100 µl		Rabbit IgG
Biotinylated Protein Ladder Detection Pack	7727	100 µl		
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml		

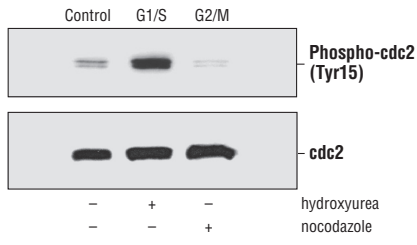
See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**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 Tyr15 and Thr14 (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 cdk7 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 phosphorylation site. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from Saos cells, either untreated or treated with hydroxyurea or nocodazole, using **Phospho-cdc2 (Tyr15) Antibody #9111** (upper) or **cdc2 Antibody #9112** (lower).

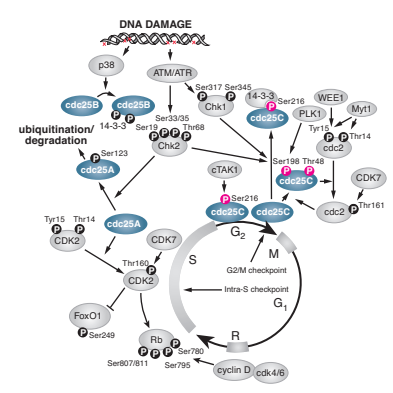
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**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.



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**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.

## 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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- Phototope<sup>®</sup>-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<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

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

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- 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<sup>®</sup> incubation and declines over the following 2 hours.