

# Phospho-cdc2 (Tyr15) Antibody

- Small 100 µl (10 Western mini-blot)
- Large 300 µl (30 Western mini-blot)

**Orders** ■ 877-616-CELL (2355)  
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, E-P Endogenous	H, M, R, Mk, X, Dr	34 kDa	Rabbit

**Background:** Entry of all eukaryotic cells into mitosis is regulated by activation of cdc2 kinase. Activation of cdc2 is controlled at several steps including cyclin binding and phosphorylation of Thr161 (1–4). However, the critical regulatory step in activating cdc2 during progression into mitosis appears to be dephosphorylation of Tyr15 and Thr14 (3,5). Phosphorylation at Tyr15 and inhibition of cdc2 is carried out by Wee1 and Myt1 protein kinases, while Tyr15 dephosphorylation and activation of cdc2 is carried out by the cdc25 phosphatase (3,4,6).

**Specificity/Sensitivity:** Phospho-cdc2 (Tyr15) Antibody detects endogenous levels of cdc2, CDK2 and CDK5 only when phosphorylated at tyrosine 15. The antibody does not cross-react with CDK4, CDK6 or CDK7. It does detect the yeast orthologue of cdc2 (cdc28) when phosphorylated at tyrosine 19.

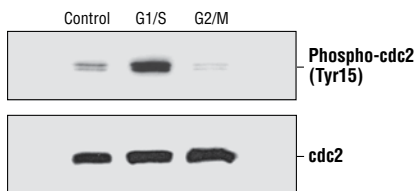
**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr15 of human cdc2. Antibodies are purified by protein A and peptide affinity chromatography.

**Selected Application References:**

- Nakajo, N. et al. (2000) Absence of Wee1 ensures the meiotic cell cycle in *Xenopus* oocytes. *Genes Dev.* 14, 328–338. Application: Western Blotting.
- Yang, H. et al. (2000) Loss of a protein phosphatase 2A regulatory subunit (Cdc55p) elicits improper regulation of swe1p degradation. *Mol. Cell. Biol.* 20, 8143–8156. Application: Western Blotting.

Misaki, K. et al. (2001) PKN delays mitotic timing by inhibition of Cdc25c: Possible involvement of PKN in the regulation of cell division. *Proc. Natl. Acad. Sci. USA* 98, 125–129. Application: Western Blotting.

Yu, C. et al. (2002) Pharmacologic mitogen-activated protein/extracellular signal-regulated kinase/mitogen-activated protein kinase inhibitors interact synergistically with STI571 to induce apoptosis in Bcr/Abl-expressing human leukemia cells. *Cancer Res.* 62, 188–199. Application: Western Blotting.



Western blot analysis of extracts from Saos cells, untreated, hydroxyurea-treated (G1/S) or nocodazole-treated (G2/M), using Phospho-cdc2 (Tyr15) Antibody (upper) or control cdc2 Antibody #9112 (lower).

Dai, Y. et al. (2001) Pharmacological inhibitors of the mitogen-activated protein kinase (MAPK) kinase/MAPK cascade interact synergistically with UCN-01 to induce mitochondrial dysfunction and apoptosis in human leukemia cells. *Cancer Res.* 61, 5106–5115. Application: Western Blotting.

Krauer, K.G. et al. (2004) The EBNA-3 gene family proteins disrupt the G2/M checkpoint. *Oncogene* 23, 1342–1353. Application: Western Blotting.

Tachibana, K. et al. (2000) c-Mos forces the mitotic cell cycle to undergo meiosis II to produce haploid gametes. *Proc. Natl. Acad. Sci. USA* 97, 14301–14306. Application: Western Blotting.

Vincent, I. et al. (1997) Aberrant expression of mitotic cdc2/cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain. *J. Neurosci.* 17, 3588–3598. Applications: IHC-FL (floating/frozen), Western Blotting.

**Background References:**

- (1) Norbury, C. and Nurse, P. (1992) *Annu. Rev. Biochem.* 61, 441–470.
- (2) Atherton-Fessler, S. et al. (1993) *Mol. Cell. Biol.* 13, 1675–1685.
- (3) Watanabe, N. et al. (1995) *EMBO J.* 14, 1878–1891.
- (4) Galaktionov, K. et al. (1995) *Genes Dev.* 9, 1046–1058.
- (5) Hunter, T. (1995) *Cell* 80, 225–236.
- (6) McGowan, C.H. and Russell, P. (1993) *EMBO J.* 12, 75–85.

**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 antibody.

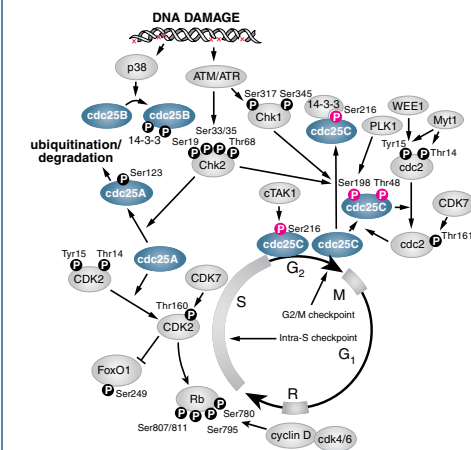
\*Species cross-reactivity is determined by Western blot.

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:100

**Companion Products:**

- Phospho-Chk1 (Ser345) Antibody #2341
- PhosphoPlus® cdc2 (Tyr15) Antibody Kit #9110
- cdc2 Antibody #9112
- cdc2 (Tyr15) Control Proteins #9113
- Phospho-cdc2 (Thr161) Antibody #9114
- cdc2 (POH1) Mouse mAb #9116
- cdc25C Antibody #9522
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003



cdc2 Signaling Pathway

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM  
Z—zebra fish B—bovine All—all species expected

## Western Immunoblotting Protocol (Primary Ab 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.

## Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

### A Solutions and Reagents

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

1. 1X Phosphate Buffered Saline (PBS)
2. **1X Cell Lysis Buffer:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , 1  $\mu\text{g}/\text{ml}$  Leupeptin

**NOTE:** CST recommends adding 1 mM PMSF before use\*.

3. **Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
4. **Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
5. **3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

### B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.

3. Remove PBS and add 0.5 ml 1X ice-cold cell lysis buffer plus 1 mM PMSF\* to each plate (10 cm) and incubate the plates on ice for 5 minutes.
4. Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate samples on ice four times for 5 seconds each.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80°C.

### C Immunoprecipitation

1. Take 200  $\mu\text{l}$  cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
2. Add either protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500  $\mu\text{l}$  of 1X cell lysis buffer. Keep on ice during washes.
4. Resuspend the pellet with 20  $\mu\text{l}$  3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes.
6. Load the sample (15–30  $\mu\text{l}$ ) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).