

# cdc25A Antibody

✓ 100 µl  
(10 Western mini-blots)

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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	H, M	70 kDa	Rabbit

**Background:** The cdc25 protein phosphatase family plays a critical role in activating cyclin-dependent kinases (CDKs) via dephosphorylation of conserved Thr14/Tyr15 inhibitory phosphorylation sites. While cdc25C is primarily responsible for activating CDK1 to overcome the G2/M checkpoint and allow mitotic entry, the primary substrate of cdc25A is CDK2, which, when active, allows progression through the G1/S and intra-S checkpoints (1). Abundance, subcellular localization and activity of cdc25A is tightly controlled by a variety of mechanisms, including phosphorylation, ubiquitination, and inhibitory binding to 14-3-3 proteins. During normal cell cycle progression, elevated c-Myc and E2F transcription factor levels lead to increased cdc25A expression (2). When conditions are favorable for DNA synthesis, cdc25A and CDK2 form an activation loop, wherein each activates the other enzyme (1). DNA damage, on the other hand, leads to multisite phosphorylation at inhibitory sites (Ser123, Ser177, Ser278, Ser292, and Thr506) by Chk1 and Chk2, which result in 14-3-3 binding and ubiquitin-mediated degradation (3,4).

**Specificity/Sensitivity:** cdc25A Antibody recognizes endogenous levels of total cdc25A protein. The antibody will cross-react with calf intestinal phosphatase (CIP) when present in abundance.

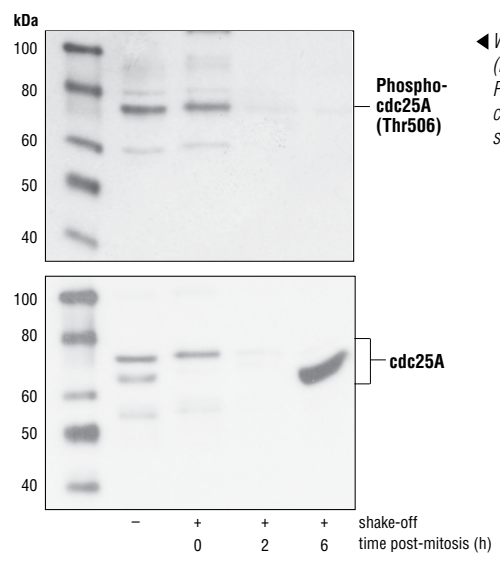
**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Ser177 of human cdc25A.

- Background References:**
- (1) Hoffmann, I. et al. (1994) *EMBO J.* 13, 4302–4310.
  - (2) Vigo, E. et al. (1999) *Mol. Cell. Biol.* 19, 6379–6395.
  - (3) Chen, M. et al. (2003) *Mol. Cell. Biol.* 23, 7488–7497.
  - (4) Sorensen, C.S. et al. (2003) *Cancer Cell* 3, 247–258.

**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.

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

- Companion Products:**
- cdc25C (5H9) Rabbit mAb #4688
  - cdc25B Antibody #9525
  - Phospho-Chk1 (Ser345) (133D3) Rabbit mAb #2348
  - Phospho-Chk2 (Thr68) Antibody #2661
  - Chk1 Kinase #7536
  - SignalSilence® Chk1 siRNA Kit (Human Specific) #6240
  - Ubiquitin (P4D1) Mouse mAb #3936
  - Anti-rabbit IgG, HRP-linked Antibody #7074
  - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
  - Prestained Protein Marker, Broad Range (Premixed Format) #7720
  - Biotinylated Protein Ladder Detection Pack #7727
  - 20X LumiGLO® Reagent and 20X Peroxide #7003



◀ Western blot analysis of extracts from asynchronous (lane 1) or synchronized (lanes 2-4) NIH/3T3 cells, using Phospho-cdc25A (Thr506) Antibody #3654 (upper) or cdc25A Antibody (lower). Cells were synchronized by mitotic shake-off and replated for the indicated times.

**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.**

## 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.