

# SignalSilence® cdc2 siRNA

✓ 10 µM in 300 µl



Cell Signaling  
TECHNOLOGY®

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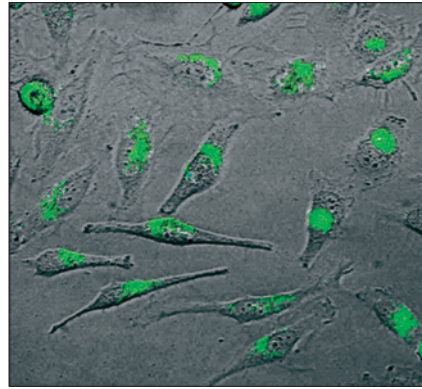
rev. 02/08/05

	Species	Molecular Wt.	Assays
cdc2 siRNA	H, (Bovine)	N/A	50–100 transfections

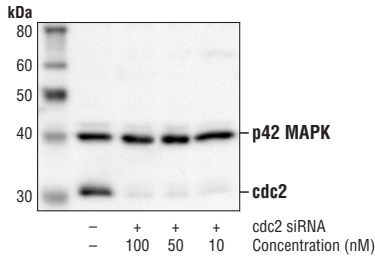
**Introduction:** SignalSilence® cdc2 siRNA from Cell Signaling Technology allows the researcher to specifically inhibit cdc2 expression using RNA interference, a method in which gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products are rigorously tested in-house and have been shown to reduce protein expression in specified cell lines.

**Directions for use:** CST recommends transfection with 50–100 nM cdc2 siRNA 48 hours prior to cell lysis. See protocol for transfection procedure.

**Tested cell lines:** HeLa



*Fluorescent detection of SignalSilence® Control siRNA (Fluorescein Conjugate) #6201 in living HeLa cells 24 hours post-transfection, demonstrating nearly 100% transfection efficiency.*



*Western blot analysis of extracts from HeLa cells, transfected with control (-) or cdc2 (+) siRNA. cdc2 was detected using cdc2 (POH1) Monoclonal Antibody #9116, and p42 was detected using p42 MAPK (3A7) Monoclonal Antibody #9107. cdc2 (POH1) Monoclonal Antibody confirms silencing of cdc2 expression, and p42 MAPK (3A7) Monoclonal Antibody is used to control for loading and specificity of cdc2 siRNA.*

**Storage:** cdc2 siRNA is supplied in RNase-free water. Aliquot and store at -20°C.

**SignalSilence® cdc2 siRNA #6376**  
Final concentration 100 nM

**Companion Products:**

SignalSilence® cdc2 siRNA Kit #6375

SignalSilence® control siRNA (Fluorescein Conjugate) #6201

cdc2 (POH1) Monoclonal Antibody #9116

Phospho-cdc2 (Tyr15) Antibody #9111

cdc2 Antibody #9112

Phospho-cdc2 (Thr161) Antibody #9114

Phototope-HRP Western Blot Detection System, Anti-mouse IgG, HRP-linked Antibody #7072

Anti-mouse IgG, HRP-linked Antibody #7076

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

LumiGLO® Reagent and Peroxide #7003



**Background:** Entry of all eukaryotic cells into M-phase of the cell cycle is regulated by activation of cdc2 kinase. Activation of cdc2 is controlled at several steps including cyclin binding and phosphorylation of threonine 161 (1-4). However, the critical regulatory step in activating cdc2 during progression into mitosis appears to be dephosphorylation of tyrosine 15 and threonine 14 (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).

RNA interference has been used to specifically silence cdc2 expression in HeLa cells, resulting in cell cycle arrest prior to spindle formation (7).

**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.
- (7) Harborth, J. et al. (2001) *J. Cell Sci.* 114, 4557-4565.

Limited Use Label License RNA interference: This product is licensed under European Patent 1144623 and foreign equivalents from Ribopharma AG, Kulmbach, Germany and is provided only for use in non-commercial research specifically excluding use by or on behalf of a commercial entity (a) in drug discovery or drug development including target identification or target validation, (b) for contract research or commercial screening services, (c) for the production or manufacture of siRNA-related products for sale, or (d) for the generation of commercial databases for sale to third parties. Information about licenses for these and other commercial uses is available from Ribopharma AG, Fritz-Hornschuch-Str. 9, D-95326 Kulmbach, Germany.

## Transfection and Western Immunoblotting Protocol

**CST recommends that researchers first confirm that the protein of interest can be detected by Western blotting in lysates from the cell type of interest.**

### siRNA Transfection Protocol:

**Use sterile technique and wear gloves to avoid cell contamination and RNA degradation.**

- A.) Day 1:** Trypsinize and plate cells to a 12-well plate in medium containing 10% serum at a density that will allow cells to reach 50% confluence on day 2.
- B.) Day 2:** (Indicated values are for a 12-well plate)
1. Remove medium from cells and replace it with 500  $\mu$ l fresh serum-containing medium.
  2. Add 100  $\mu$ l of serum-free medium to a clean, sterile microfuge tube.
  3. Add 2  $\mu$ l of Transfection Reagent to the tube. Mix by pipetting up and down.
  4. Incubate at room temperature for 5 minutes.
  5. Add the appropriate volume of siRNA (stocks are 10  $\mu$ M in RNase-free water) to the tube. For example, add 6  $\mu$ l of 10  $\mu$ M stock siRNA to the microfuge tube to yield a final concentration of 100 nM, or 3  $\mu$ l to yield a concentration of 50 nM, when the mixture is added to the well containing 500  $\mu$ l. See data sheet for recommended final siRNA concentration. Mix by pipetting up and down gently.
  6. Incubate for 5 minutes at room temperature.
  7. Add 100  $\mu$ l of the mixture to the well containing 500  $\mu$ l medium all at once (not drop-wise).
  8. Agitate vigorously to disperse siRNA evenly, but avoid spillage of medium from one well to another.
- C.) Day 3:** Replace the medium with fresh medium. Examine fluorescein-labeled non-specific siRNA-transfected cells using a fluorescence microscope to determine transfection efficiency. For a 24 hour time point, proceed to step "D".
- D.) Day 4 (48 hour time point):**  
To prepare cell lysates for Western blot analysis, proceed to step 2 of Protein Blotting protocol. CST recommends that researchers perform a preliminary Western blot using control (non-targeted) antibody to detect protein from approximately 7  $\mu$ l of each cell lysate to confirm that there is an equal concentration of cellular protein in each sample.

### Solutions and Reagents

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

#### Transfer Buffer:

25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

#### SDS Sample Buffer (1X):

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

#### 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%).

#### 10X TBS (Tris-buffered saline):

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).

#### Primary Antibody Dilution Buffer:

1X TBS, 0.1% Tween-20 with 5% BSA (for polyclonal antibodies) or 5% nonfat dry milk (for monoclonal antibodies or a combination of a polyclonal and a monoclonal antibody); for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA or nonfat dry milk and mix well. While stirring, add 20  $\mu$ l Tween-20 (100%).

#### Phototope<sup>®</sup>-HRP Western Blot Detection:

Biotinylated protein marker, secondary antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>™</sup> chemiluminescent reagent, peroxide

#### Wash Buffer TBS/T:

1X TBS, 0.1% Tween-20

### Blotting Membrane

This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

### Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells twice with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (50  $\mu$ l per well of 12-well plate). Immediately scrape the cells off the plate and transfer the extract to a microfuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20  $\mu$ l sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20  $\mu$ l onto SDS-PAGE gel (10 cm x 10 cm).

*Note: CST recommends loading prestained molecular weight markers (#7720, 10  $\mu$ l/lane) to verify electrotransfer and biotinylated protein markers (#7727, 10  $\mu$ l/lane) to determine molecular weights.*

8. Electrotransfer to nitrocellulose membrane.

**For Western blots, incubate membrane with diluted antibody in 5% BSA, (for polyclonal antibodies) or 5% nonfat dry milk (for monoclonal antibodies or a combination of a polyclonal and a monoclonal antibody). 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

## Western Immunoblotting Protocol

### 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.*

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody with loading control antibody (at the appropriate dilution) in 10 ml Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (dilution varies with manufacturer) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers (if using) in 10 ml of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 ml of TBS/T.

### Detection of Proteins

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

*Note: LumiGLO™ Substrate can be further diluted if signal response is too fast.*

2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

*Note: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO™ incubation and declines over the following 2 hours.*