

SignalSilence® cdc25C siRNA (Human Specific)



Cell Signaling
TECHNOLOGY®

✓ 10 µM in 300 µl

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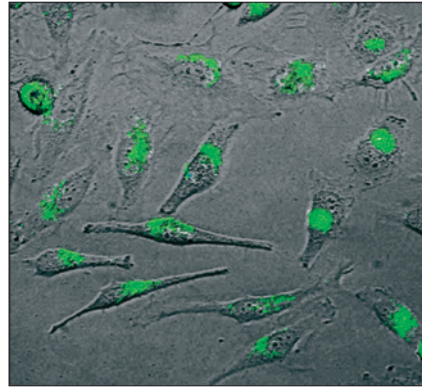
rev. 01/20/05

	Species	Molecular Wt.	Assays
cdc25C siRNA	H	N/A	50–100 transfections

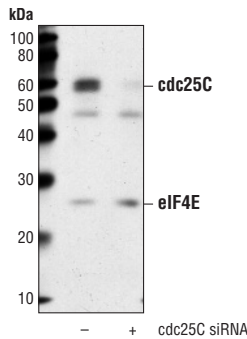
Introduction: SignalSilence® cdc25C siRNA from Cell Signaling Technology allows the researcher to specifically inhibit cdc25C expression using RNA interference, a method in which gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® 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 100 nM human-specific cdc25C siRNA 48 hours prior to cell lysis. See Protocol for transfection procedure.

Tested cell lines: HeLa, M059K, M059J



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 cdc25C (+) siRNA. Cdc25C was detected using a cdc25C antibody, eIF4E was detected using eIF4E Antibody #9742. The cdc25C antibody confirms silencing of cdc25C expression, and the eIF4E Antibody is used to control for loading and specificity of cdc25C siRNA.

Storage: cdc25C siRNA is supplied in RNase-free water. Aliquot and store at -20°C.

SignalSilence® cdc25C siRNA (Human Specific) #6291
Final concentration 100 nM

Companion Products:
SignalSilence® Control siRNA (Fluorescein Conjugate) #6201

SignalSilence® cdc25C siRNA Kit (Human Specific) #6290

Phospho-cdc25C (Thr48) Antibody #9527

Phospho-cdc25C (Ser216) Antibody #9528

Phototope®-HRP Western Detection System:
Anti-rabbit IgG, HRP-linked Antibody #7074

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

Biotinylated Protein Ladder Detection Pack #7727

LumiGLO® Reagent and Peroxide #7003



Background: cdc25 is a protein phosphatase responsible for dephosphorylating and activating cdc2, a crucial step in regulating the entry of all eukaryotic cells into the M-phase of the cell cycle (1). cdc25C is constitutively phosphorylated at Ser216 throughout interphase by c-TAK1, while phosphorylation at this site is DNA damage-dependent at the G2/M checkpoint (2). Ser216-phosphorylated cdc25C binds to members of the 14-3-3 family of proteins, sequestering cdc25C in the cytoplasm to prevent premature mitosis (3). The checkpoint kinases Chk1 and Chk2 have been shown to phosphorylate cdc25C at Ser216 in response to DNA damage (4,5).

Small interfering RNA (siRNA) has been used to specifically silence cdc25C expression in HeLa cells, causing a G1/S checkpoint arrest (6).

Background References:

- (1) Jessus, C. and Ozon, R. (1995) *Prog. Cell Cycle Res.* 1, 215–228.
- (2) Peng, C.Y. et al. (1997) *Science* 277, 1501–1505.
- (3) Kumagai, A. and Dunphy, W.G. (1999) *Genes Dev.* 13, 1067–1072.
- (4) Blasina, A. et al. (1999) *Curr. Biol.* 9, 1–10.
- (5) Furnari, B. et al. (1999) *Mol. Biol. Cell* 10, 833–845.
- (6) Turowski, P. et al. (2003) *Mol. Biol. Cell* 14, 2984–2998.

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 μ l fresh serum-containing medium.
 2. Add 100 μ l of serum-free medium to a clean, sterile microfuge tube.
 3. Add 2 μ 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 μ M in RNase-free water) to the tube. For example, add 6 μ l of 10 μ M stock siRNA to the microfuge tube to yield a final concentration of 100 nM, or 3 μ l to yield a concentration of 50 nM, when the mixture is added to the well containing 500 μ 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 μ l of the mixture to the well containing 500 μ 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 μ 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 μ l Tween-20 (100%).

Phototope®-HRP Western Blot Detection:

Biotinylated protein marker, secondary antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO™ 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 μ 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 μ l sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 μ l onto SDS-PAGE gel (10 cm x 10 cm).

Note: CST recommends loading prestained molecular weight markers (#7720, 10 μ l/lane) to verify electrotransfer and biotinylated protein markers (#7727, 10 μ 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 cm²) 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.