

# SignalSilence® ILK1 siRNA Kit

1 Kit  
 (50 Transfections per siRNA)

**Orders** ■ 877-616-CELL (2355)  
 orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
 info@cellsignal.com  
**Web** ■ www.cellsignal.com

New 11/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.  
 This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Isotype	Assay
SignalSilence® ILK1 siRNA I	6202	10 µM in 150 µl		50 Transfections
SignalSilence® ILK1 siRNA II	6528	10 µM in 150 µl		50 Transfections
ILK1 (4G9) Rabbit mAb	3856	40 µl	Rabbit IgG	4 Western mini blots

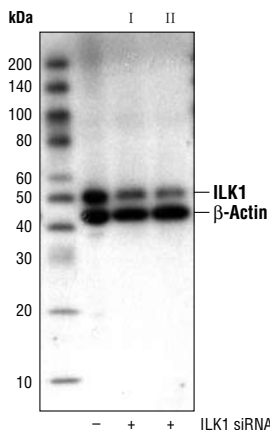
### Species Cross-Reactivity: H

### Molecular Weight of Protein: 51 kDa

**Description:** SignalSilence® ILK1 siRNA Kit from Cell Signaling Technology (CST) allows the researcher to specifically inhibit ILK expression. The kit utilizes RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA kits are rigorously tested in-house and have been shown to reduce target protein expression. SignalSilence® ILK1 siRNA Kit includes ILK1 siRNA I and II and a target-specific ILK1 antibody to confirm the silencing of ILK1 expression by western analysis.

**Background:** Integrin-linked kinases (ILKs) couple integrins and growth factors to downstream pathways involved in cell survival, cell cycle control, cell-cell adhesion and cell motility (1). ILK functions as a scaffold bridging the extracellular matrix (ECM) and growth factor receptors to the actin cytoskeleton through interactions with integrin, PINCH (which links ILK to the RTKs via Nck2), CH-ILKBP and af-ixin (1). ILK phosphorylates Akt at Ser473, GSK-3 on Ser9, myosin light chain 2 (MLC2) on Ser18/Thr19, as well as af-ixin (2-5). These phosphorylation events are key regulatory steps in modulating the activities of the targets. ILK activity is stimulated by PI3 kinase and negatively regulated by the tumor suppressor PTEN and a PP2C protein phosphatase, ILKAP (1,3,6). It has been suggested that the conserved Ser343 residue in the activation loop plays a key role in the activation of ILK1 (2).

**Directions for Use:** CST recommends transfection with 100 nM ILK siRNA 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Fluorescein Conjugate) #6201 (-), SignalSilence® ILK1 siRNA I (+) or SignalSilence® ILK1 siRNA II (+), using ILK1 (4G9) Rabbit mAb and beta-Actin Antibody #4967. ILK1 (4G9) Rabbit mAb confirms silencing of ILK1 expression, while the beta-Actin Antibody is used to control for loading and specificity of ILK1 siRNA.

**Entrez-Gene ID** #3611  
**Swiss-Prot Acc.** #Q13418

**Storage:** ILK1 siRNA I and II are supplied in RNase-free water. Aliquot and store at -20°C. Antibody is 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:

- SignalSilence® Control siRNA (Fluorescein Conjugate) #6201
- SignalSilence® Control siRNA (Unconjugated) #6568
- ILK1 Antibody #3862
- Biotinylated Protein Ladder Detection Pack #7727
- Anti-rabbit IgG, HRP-linked Antibody #7074

### Background References:

- (1) Wu, C. and Dedhar, S. (2000) *J. Biol. Chem.* 155, 505–510.
- (2) Persad, S. et al. (2001) *J. Biol. Chem.* 276, 27462–27469.
- (3) Persad, S. et al. (2000) *J. Cell Biol.* 153, 1161–1173.
- (4) Deng, J.T. et al. (2001) *J. Biol. Chem.* 276, 16365–16373.
- (5) Yamaji, S. et al. (2001) *J. Cell Biol.* 153, 1251–1264.
- (6) Morimoto, A.M. et al. (2000) *Oncogene* 19, 200–209.

## 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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **Phototope®-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® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. 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.

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 three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

### D 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 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® incubation and declines over the following 2 hours.