

# SignalSilence® Pool p42 MAPK siRNA

✓ 10 µM in 60 µl



Cell Signaling  
TECHNOLOGY®

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

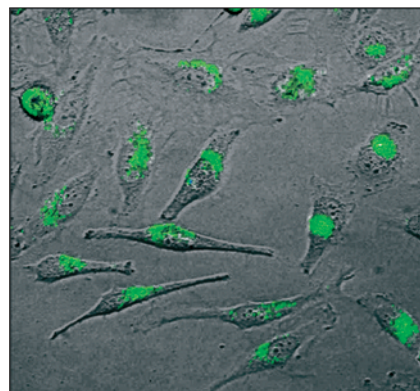
	Species	Molecular Wt.	Assays
Pool p42 MAPK siRNA	H, Mk	N/A	50–100 transfections

**Introduction:** SignalSilence® Pool p42 MAP Kinase siRNA from Cell Signaling Technology allows the researcher to specifically inhibit p42 MAP Kinase 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. The p42 MAP Kinase siRNA is a heterogeneous mixture of 21–22 bp siRNAs. A 283 bp cDNA template of human p42 MAP Kinase was transcribed by T7 RNA polymerase to create double-stranded RNA (dsRNA). RNase III was used to cleave the dsRNA in the presence of Mn<sup>2+</sup> buffer to 21–22 bp siRNA. 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 5 to 20 nM pool p42 MAP Kinase siRNA 48 to 72 hours prior to cell lysis. See Protocol for transfection procedure.

**Tested cell lines:** HeLa

**Background:** Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (ERK1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines (1–3) and is an important target in the diagnosis and treatment of cancer (4). Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK), and a MAP kinase. While multiple ERK1/2 MAP3Ks have been identified, including the Raf family, Mos, and Tpl2/Cot, MEK1 and MEK2 are the primary MAPKKs in this pathway (5,6). MEK1 and MEK2 activate ERK1/p44 and ERK2/p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively. Several downstream targets of ERK1/2 have been identified, including p90RSK (7) and the transcription factor Elk-1 (8,9). ERK1/2 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs (10), along with MEK inhibitors such as U0126 and PD98059.



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

**Background References:**

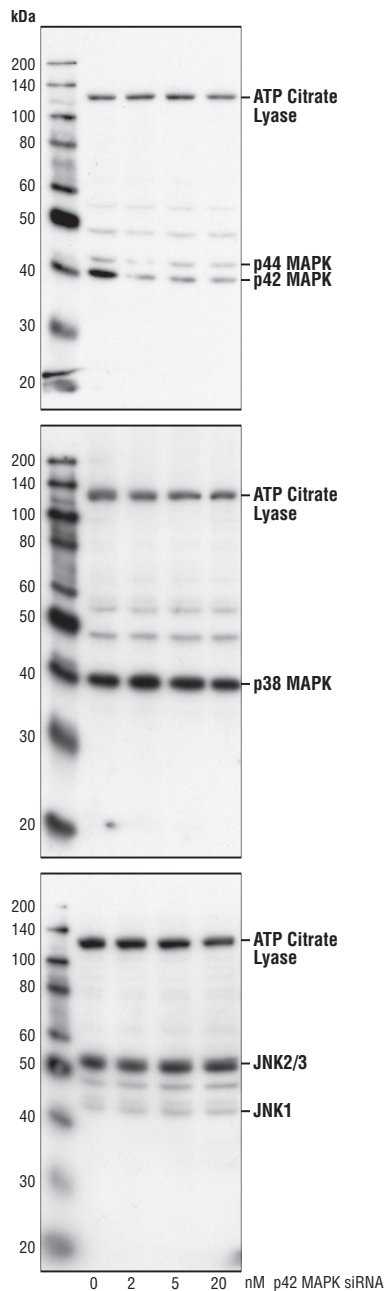
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- (2) Baccarini, M. (2005) *FEBS Lett* 579, 3271–7.
- (3) Meloche, S. and Pouysségur, J. (2007) *Oncogene* 26, 3227–39.
- (4) Roberts, P.J. and Der, C.J. (2007) *Oncogene* 26, 3291–310.
- (5) Rubinfeld, H. and Seger, R. (2005) *Mol Biotechnol* 31, 151–74.
- (6) Murphy, L.O. and Blenis, J. (2006) *Trends Biochem Sci* 31, 268–75.
- (7) Dalby, K.N. et al. (1998) *J Biol Chem* 273, 1496–505.
- (8) Marais, R. et al. (1993) *Cell* 73, 381–93.
- (9) Kortenjann, M. et al. (1994) *Mol Cell Biol* 14, 4815–24.
- (10) Owens, D.M. and Keyse, S.M. (2007) *Oncogene* 26, 3203–13.

**Storage:** Pool p42 siRNA is supplied in RNase-free water. Aliquot and store at -20°C.

**SignalSilence® Pool p42 MAPK siRNA #6391**  
Final concentration 5–20 nM

**Companion Products:**

- SignalSilence® Pool p42 MAPK siRNA Kit #6390
- SignalSilence® Control siRNA (Fluorescein Conjugate) #6201
- Erk5 Antibody #3372
- Phospho-p44/42 MAPK (Thr202/Tyr204) (D13.14.4E) Rabbit mAb #4370
- Phospho-p44/42 MAP Kinase (Thr202/Tyr204) Antibody #9101
- p44/42 MAP Kinase Antibody #9102
- SignalSilence® MEK1 siRNA Kit (Human Specific) #6425
- SignalSilence® MEK1 siRNA (Human Specific) #6426
- SignalSilence® MEK2 siRNA Kit (Human Specific) #6430
- SignalSilence® MEK2 siRNA (Human Specific) #6431
- SignalSilence® p44 MAPK siRNA Kit (Human Specific) #6435
- SignalSilence® p44 MAPK siRNA (Human Specific) #6436



◀ Western blot analysis of extracts from HeLa cells transfected with 20 nM control siRNA #6201 or 2, 5, or 20 nM pool 42 MAPK siRNA, using p44/42 MAP Kinase Antibody #9102 (upper), p38 MAP Kinase Antibody #9212 (middle) and SAPK/JNK (56G8) Rabbit mAb #9258 (lower) in combination with ATP-Citrate Lyase Antibody #4332. The 44/42 MAP Kinase Antibody confirms silencing of p42 MAP Kinase expression, and ATP-Citrate Lyase Antibody is used to control for loading and specificity of pool p42 MAP Kinase siRNA. The experiment also demonstrates that the pool p42 MAPK siRNA does not interfere with the expression of p44 MAPK, p38 MAPK or SAPK/JNKs.

## 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®-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  $\mu$ 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.*