

#6630 Store at -20°C

# SignalSilence® AMPK $\alpha$ 2 siRNA II



✓ 10  $\mu$ M in 300  $\mu$ l  
(100 transfections)

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**For Research Use Only. Not For Use In Diagnostic Procedures.**

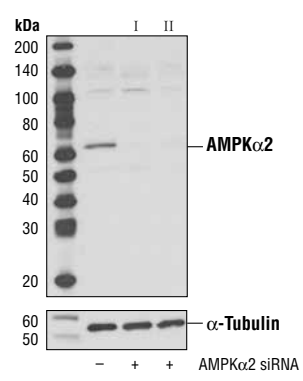
### Species Cross-Reactivity: H

**Description:** SignalSilence® AMPK $\alpha$ 2 siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit AMPK $\alpha$ 2 expression using 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 products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

**Background:** AMP-activated protein kinase (AMPK) is highly conserved from yeast to plants and animals and plays a key role in the regulation of energy homeostasis (1). AMPK is a heterotrimeric complex composed of a catalytic  $\alpha$  subunit and regulatory  $\beta$  and  $\gamma$  subunits, each of which is encoded by two or three distinct genes ( $\alpha$ 1, 2;  $\beta$ 1, 2;  $\gamma$ 1, 2, 3) (2). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia and ischemia (1). The tumor suppressor LKB1, in association with accessory proteins STRAD and MO25, phosphorylates AMPK $\alpha$  at Thr172 in the activation loop and this phosphorylation is required for AMPK activation (3-5). AMPK $\alpha$  is also phosphorylated at Thr258 and Ser485 (for  $\alpha$ 1; Ser491 for  $\alpha$ 2). The upstream kinase and the biological significance of these phosphorylation events have yet to be elucidated (6). The  $\beta$ 1 subunit is post-translationally modified by myristoylation and multi-site phosphorylation including Ser24/25, Ser96, Ser101, Ser108 and Ser182 (6,7). Phosphorylation at Ser108 of the  $\beta$ 1 subunit seems to be required for the activation of AMPK enzyme, while phosphorylation at Ser24/25 and Ser182 affects AMPK localization (7). Several mutations in AMPK $\gamma$  subunits have been identified, most of which are located in the putative AMP/ATP binding sites (CBS or Bateman domains). Mutations at these sites lead to reduction of AMPK activity and cause glycogen accumulation in heart or skeletal muscle (1,2). Accumulating evidence indicates that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS (1).

**Directions for Use:** CST recommends transfection with 100 nM AMPK $\alpha$ 2 siRNA II 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.

**Quality Control:** Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® AMPK $\alpha$ 2 siRNA I #6620 (+) or SignalSilence® AMPK $\alpha$ 2 siRNA II (+), using AMPK $\alpha$ 2 Antibody #2757 (upper) or  $\alpha$ -Tubulin (11H10) Rabbit mAb #2125 (lower). The AMPK $\alpha$ 2 Antibody confirms silencing of AMPK $\alpha$ 2 expression, while the  $\alpha$ -Tubulin (11H10) Rabbit mAb is used as a loading control.

**Entrez-Gene ID** #5563  
**Swiss-Prot Acc.** #P54646

**Storage:** AMPK $\alpha$ 2 siRNA II is supplied in RNase-free water. Aliquot and store at -20°C.

**Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.**

### Background References:

- (1) Hardie, D.G. (2004) *J Cell Sci* 117, 5479-87.
- (2) Carling, D. (2004) *Trends Biochem Sci* 29, 18-24.
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- (4) Lizzano, J.M. et al. (2004) *EMBO J* 23, 833-43.
- (5) Shaw, R.J. et al. (2004) *Proc Natl Acad Sci USA* 101, 3329-35.
- (6) Woods, A. et al. (2003) *J Biol Chem* 278, 28434-42.
- (7) Warden, S.M. et al. (2001) *Biochem J* 354, 275-83.

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**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA—Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine  
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.