

# AMPK $\alpha$ 1 siRNA (m): sc-29674

## BACKGROUND

AMPK (for 5'-AMP-activated protein kinase) is a heterotrimeric complex comprising a catalytic  $\alpha$  subunit and regulatory  $\beta$  and  $\gamma$  subunits. It protects cells from stresses that cause ATP depletion by switching off ATP-consuming biosynthetic pathways. AMPK is activated by high AMP and low ATP through a mechanism involving allosteric regulation, promotion of phosphorylation by an upstream protein kinase known as AMPK kinase, and inhibition of dephosphorylation. Activated AMPK can phosphorylate and regulate *in vivo* hydroxy-methylglutaryl-CoA reductase and acetyl-CoA carboxylase, which are key regulatory enzymes of sterol synthesis and fatty acid synthesis, respectively. The human AMPK $\alpha$ 1 and AMPK $\alpha$ 2 genes encode 548 amino acid and 552 amino acid proteins, respectively. Human AMPK $\beta$ 1 encodes a 271 amino acid protein and human AMPK $\beta$ 2 encodes a 272 amino acid protein. The human AMPK $\gamma$ 1 gene encodes a 331 amino acid protein. Human AMPK $\gamma$ 2 and AMPK $\gamma$ 3, which are 569 and 492 amino acid proteins, respectively, contain unique N-terminal domains and may participate directly in the binding of AMP within the AMPK complex.

## REFERENCES

1. Stapleton, D., et al. 1996. Mammalian AMP-activated protein kinase subfamily. *J. Biol. Chem.* 271: 611-614.
2. Stapleton, D., et al. 1997. AMP-activated protein kinase isoenzyme family: subunit structure and chromosomal location. *FEBS Lett.* 409: 452-456.

## CHROMOSOMAL LOCATION

Genetic locus: Prkaa1 (mouse) mapping to 15 A1.

## PRODUCT

AMPK $\alpha$ 1 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AMPK $\alpha$ 1 shRNA Plasmid (m): sc-29674-SH and AMPK $\alpha$ 1 shRNA (m) Lentiviral Particles: sc-29674-V as alternate gene silencing products.

For independent verification of AMPK $\alpha$ 1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29674A, sc-29674B and sc-29674C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

AMPK $\alpha$ 1 siRNA (m) is recommended for the inhibition of AMPK $\alpha$ 1 expression in mouse cells.

## SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10  $\mu$ M in 66  $\mu$ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

## GENE EXPRESSION MONITORING

AMPK $\alpha$ 1 (H-4): sc-398861 is recommended as a control antibody for monitoring of AMPK $\alpha$ 1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AMPK $\alpha$ 1 gene expression knockdown using RT-PCR Primer: AMPK $\alpha$ 1 (m)-PR: sc-29674-PR (20  $\mu$ l, 481 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Hattori, Y., et al. 2006. Metformin inhibits cytokine-induced nuclear factor  $\kappa$ B activation via AMP-activated protein kinase activation in vascular endothelial cells. *Hypertension* 47: 1183-1188.
2. Finocchietto, P.V., et al. 2011. Defective leptin-AMP-dependent kinase pathway induces nitric oxide release and contributes to mitochondrial dysfunction and obesity in ob/ob mice. *Antioxid. Redox Signal.* 15: 2395-2406.
3. Kalderon, B., et al. 2012. Suppression of adipose lipolysis by long-chain fatty acid analogs. *J. Lipid Res.* 53: 868-878.
4. Siddique, M.M., et al. 2013. Ablation of dihydroceramide desaturase 1, a therapeutic target for the treatment of metabolic diseases, simultaneously stimulates anabolic and catabolic signaling. *Mol. Cell. Biol.* 33: 2353-2369.
5. Xie, Z., et al. 2014. Silibinin activates AMP-activated protein kinase to protect neuronal cells from oxygen and glucose deprivation-re-oxygenation. *Biochem. Biophys. Res. Commun.* 454: 313-319.
6. Velagapudi, R., et al. 2017. AMPK and SIRT1 activation contribute to inhibition of neuroinflammation by thymoquinone in BV2 microglia. *Mol. Cell. Biochem.* 435: 149-162.
7. Zhang, Y., et al. 2018. TFEB-dependent induction of thermogenesis by the hepatocyte SLC2A inhibitor trehalose. *Autophagy* 14: 1959-1975.
8. Liang, C., et al. 2019. MiR-451 antagonist protects against cardiac fibrosis in streptozotocin-induced diabetic mouse heart. *Life Sci.* 224: 12-22.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.