

# AMPK $\alpha$ 2 siRNA (h): sc-38923

## 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* hydroxymethylglutaryl-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: PRKAA2 (human) mapping to 1p32.2.

## PRODUCT

AMPK $\alpha$ 2 siRNA (h) 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 $\alpha$ 2 shRNA Plasmid (h): sc-38923-SH and AMPK $\alpha$ 2 shRNA (h) Lentiviral Particles: sc-38923-V as alternate gene silencing products.

For independent verification of AMPK $\alpha$ 2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-38923A, sc-38923B and sc-38923C.

## 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 $\alpha$ 2 siRNA (h) is recommended for the inhibition of AMPK $\alpha$ 2 expression in human 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 $\alpha$ 1/2 (D-6): sc-74461 is recommended as a control antibody for monitoring of AMPK $\alpha$ 2 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 $\alpha$ 2 gene expression knockdown using RT-PCR Primer: AMPK $\alpha$ 2 (h)-PR: sc-38923-PR (20  $\mu$ l, 577 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Hou, C.H., et al. 2008. AMP-activated protein kinase is involved in COX-2 expression in response to ultrasound in cultured osteoblasts. *Cell. Signal.* 20: 978-988.
2. Nerstedt, A., et al. 2010. AMP-activated protein kinase inhibits IL-6-stimulated inflammatory response in human liver cells by suppressing phosphorylation of signal transducer and activator of transcription 3 (STAT3). *Diabetologia* 53: 2406-2416.
3. Tong, K.M., et al. 2011. Adiponectin increases MMP-3 expression in human chondrocytes through adipon1 signaling pathway. *J. Cell. Biochem.* 112: 1431-1440.
4. Nerstedt, A., et al. 2013. Pharmacological activation of AMPK suppresses inflammatory response evoked by IL-6 signalling in mouse liver and in human hepatocytes. *Mol. Cell. Endocrinol.* 375: 68-78.
5. Hwang, H.J., et al. 2015. The dipeptidyl peptidase-IV inhibitor inhibits the expression of vascular adhesion molecules and inflammatory cytokines in HUVECs via Akt- and AMPK-dependent mechanisms. *Mol. Cell. Endocrinol.* 405: 25-34.
6. Georgiadou, M., et al. 2017. AMPK negatively regulates tensin-dependent integrin activity. *J. Cell Biol.* 216: 1107-1121.
7. Johnson, J., et al. 2020. Targeting PI3K and AMPK $\alpha$  signaling alone or in combination to enhance radiosensitivity of triple negative breast cancer. *Cells* 9: E1253.

## RESEARCH USE

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