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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

AMPK α 1 siRNA (h2): sc-44281

BACKGROUND

AMPK (for 5'-AMP-activated protein kinase) is a heterotrimeric complex comprising a catalytic α subunit and regulatory β and γ 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 α 1 and AMPK α 2 genes encode 548 amino acid and 552 amino acid proteins, respectively. Human AMPK β 1 encodes a 271 amino acid protein and human AMPK β 2 encodes a 272 amino acid protein. The human AMPK γ 1 gene encodes a 331 amino acid protein. Human AMPK γ 2 and AMPK γ 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.

CHROMOSOMAL LOCATION

Genetic locus: PRKAA1 (human) mapping to 5p13.1.

PRODUCT

AMPK α 1 siRNA (h2) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AMPK α 1 shRNA Plasmid (h2): sc-44281-SH and AMPK α 1 shRNA (h2) Lentiviral Particles: sc-44281-V as alternate gene silencing products.

For independent verification of AMPK α 1 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44281A, sc-44281B and sc-44281C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ C, avoid contact with RNAses and repeated freeze thaw cycles.

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

APPLICATIONS

AMPK α 1 siRNA (h2) is recommended for the inhibition of AMPK α 1 expression in human cells.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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 μ M in 66 μ 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 α 1 (H-4): sc-398861 is recommended as a control antibody for monitoring of AMPK α 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).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AMPK α 1 gene expression knockdown using RT-PCR Primer: AMPK α 1 (h2)-PR: sc-44281-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

- Okayasu, T., et al. 2008. PPAR α activators upregulate eNOS activity and inhibit cytokine-induced NF κ B activation through AMP-activated protein kinase activation. *Life Sci.* 82: 884-891.
- Bair, A.M., et al. 2009. Ca²⁺ entry via TRPC channels is necessary for thrombin-induced NF κ B activation in endothelial cells through AMP-activated protein kinase and protein kinase C δ . *J. Biol. Chem.* 284: 563-574.
- Huang, C.Y., et al. 2010. Adiponectin increases BMP-2 expression in osteoblasts via AdipoR receptor signaling pathway. *J. Cell. Physiol.* 224: 475-483.
- Tomizawa, A., et al. 2011. Fenofibrate suppresses microvascular inflammation and apoptosis through adenosine monophosphate-activated protein kinase activation. *Metab. Clin. Exp.* 60: 513-522.

RESEARCH USE

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