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AMPK α 1 siRNA (r): sc-270142

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.

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 (rat) mapping to 2q16.

PRODUCT

AMPK α 1 siRNA (r) 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 (r): sc-270142-SH and AMPK α 1 shRNA (r) Lentiviral Particles: sc-270142-V as alternate gene silencing products.

For independent verification of AMPK α 1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270142A, sc-270142B and sc-270142C.

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 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 (r) is recommended for the inhibition of AMPK α 1 expression in rat 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 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 1/2 (D-6): sc-74461 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).

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 (r)-PR: sc-270142-PR (20 μ l, 552 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. Activation of AMP-activated protein kinase enhances Angiotensin II-induced proliferation in cardiac fibroblasts. *Hypertension* 47: 265-270.
2. Kim, J.Y., et al. 2013. *In vivo* activating transcription factor 3 silencing ameliorates the AMPK compensatory effects for ER stress-mediated β -cell dysfunction during the progression of type-2 diabetes. *Cell. Signal.* 25: 2348-2361.
3. Kamga Pride, C., et al. 2014. Nitrite activates protein kinase A in normoxia to mediate mitochondrial fusion and tolerance to ischaemia/reperfusion. *Cardiovasc. Res.* 101: 57-68.
4. Than, A., et al. 2015. Apelin enhances brown adipogenesis and browning of white adipocytes. *J. Biol. Chem.* 290: 14679-14691.
5. Duan, P., et al. 2017. 4-nonylphenol induces autophagy and attenuates mTOR-p70S6K/4EBP1 signaling by modulating AMPK activation in Sertoli cells. *Toxicol. Lett.* 267: 21-31.
6. Asensio-Lopez, M.D.C., et al. 2018. Pharmacological inhibition of the mitochondrial NADPH oxidase 4/PKC α /Gal-3 pathway reduces left ventricular fibrosis following myocardial infarction. *Transl. Res.* 199: 4-23.
7. Lee, Y.J., et al. 2019. Cilostazol protects hepatocytes against alcohol-induced apoptosis via activation of AMPK pathway. *PLoS ONE* 14: e0211415.

RESEARCH USE

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