SANTA CRUZ BIOTECHNOLOGY, INC.

AMPKα1 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 AMPKα1 and AMPKα2 genes encode 548 amino acid and 552 amino acid proteins, respectively. Human AMPKβ1 encodes a 271 amino acid protein and human AMPKβ2 encodes a 272 amino acid protein. The human AMPKγ1 gene encodes a 331 amino acid protein. Human AMPKγ2 and AMPKγ3, 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.
3. Hardie, D.G., et al. 1997. The AMP-activated protein kinase-fuel gauge of the mammalian cell? Eur. J. Biochem. 246: 259-273.

CHROMOSOMAL LOCATION

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

PRODUCT

AMPKα1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AMPKα1 shRNA Plasmid (h2): sc-44281-SH and AMPKα1 shRNA (h2) Lentiviral Particles: sc-44281-V as alternate gene silencing products.

For independent verification of AMPKα1 (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° 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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

AMPKα1 siRNA (h2) is recommended for the inhibition of AMPKα1 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 µM in 66 µl. 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

AMPKα1 (H-4): sc-398861 is recommended as a control antibody for monitoring of AMPKα1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 AMPKα1 gene expression knockdown using RT-PCR Primer: AMPKα1 (h2)-PR: sc-44281-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Okayasu, T., et al. 2008. PPARα activators upregulate eNOS activity and inhibit cytokine-induced NFκB activation through AMP-activated protein kinase activation. Life Sci. 82: 884-891.
2. Bair, A.M., et al. 2009. Ca^{2+} entry via TRPC channels is necessary for thrombin-induced NFκB activation in endothelial cells through AMP-activated protein kinase and protein kinase Cδ. J. Biol. Chem. 284: 563-574.
3. Huang, C.Y., et al. 2010. Adiponectin increases BMP-2 expression in osteoblasts via AdipoR receptor signaling pathway. J. Cell. Physiol. 224: 475-483.
4. 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.