

# AMPK $\alpha$ 1 siRNA (bovine): sc-270395

## 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. AMPK $\alpha$ 1, also known as PRKAA1 (protein kinase, AMP-activated,  $\alpha$ 1 catalytic subunit) is a 458 amino acid bovine protein that is encoded by a gene that maps to chromosome 20. The human homolog maps to human chromosome 5.

## 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.
4. Thornton, C., et al. 1998. Identification of a novel AMP-activated protein kinase  $\beta$  subunit isoform that is highly expressed in skeletal muscle. *J. Biol. Chem.* 273: 12443-12450.

## CHROMOSOMAL LOCATION

Genetic locus: PRKAA1 (bovine) mapping to 20.

## PRODUCT

AMPK $\alpha$ 1 siRNA (bovine) 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 (bovine): sc-270395-SH and AMPK $\alpha$ 1 shRNA (bovine) Lentiviral Particles: sc-270395-V as alternate gene silencing products.

For independent verification of AMPK $\alpha$ 1 (bovine) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270395A, sc-270395B and sc-270395C.

## 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 (bovine) is recommended for the inhibition of AMPK $\alpha$ 1 expression in bovine 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 $\alpha$ 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 $\alpha$ 1 gene expression knockdown using RT-PCR Primer: AMPK $\alpha$ 1 (bovine)-PR: sc-270395-PR (20  $\mu$ l, 636 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Teng, R.J., et al. 2013. AMP kinase activation improves angiogenesis in pulmonary artery endothelial cells with in utero pulmonary hypertension. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 304: L29-L42.
2. Gu, X., et al. 2018. AMPK/SIRT1/p38 MAPK signaling pathway regulates alcohol-induced neurodegeneration by resveratrol. *Mol. Med. Rep.* 17: 5402-5408.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.