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- Trockeneiszuschlag
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- Expressversand

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# AMPK $\alpha$ 1/2 shRNA (h) Lentiviral Particles: sc-45312-V

## 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.
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, PRKAA2 (human) mapping to 1p32.2.

## PRODUCT

AMPK $\alpha$ 1/2 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see AMPK $\alpha$ 1/2 siRNA (h): sc-45312 and AMPK $\alpha$ 1/2 shRNA Plasmid (h): sc-45312-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ\text{C}$ . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ\text{C}$  for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

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

## APPLICATIONS

AMPK $\alpha$ 1/2 shRNA (h) Lentiviral Particles is recommended for the inhibition of AMPK $\alpha$ 1 and AMPK $\alpha$ 2 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

AMPK $\alpha$ 1/2 (D-6): sc-74461 is recommended as a control antibody for monitoring of AMPK $\alpha$ 1 and 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).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AMPK $\alpha$ 1 and AMPK $\alpha$ 2 gene expression knockdown using RT-PCR Primer: AMPK $\alpha$ 1/2 (h)-PR: sc-45312-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.