

AMPD1 siRNA (h): sc-78635

BACKGROUND

Adenosine monophosphate (AMP) deaminase is a cytosolic enzyme responsible for the hydrolytic deamination of AMP to inosine monophosphate (IMP) and NH_3 . AMP deaminase functions as a homotetramer and participates in the purine nucleotide cycle, playing an important role in energy metabolism. Three differentially expressed isozymes of AMP deaminase exist in mammals, namely AMPD1, AMPD2 and AMPD3, and they differ among their N-terminal domains while sharing a conserved C-terminal catalytic domain. AMPD1 is expressed in skeletal muscle; AMPD2 is found in undifferentiated myoblasts, smooth muscle, embryonic muscle and non-muscle tissue; and AMPD3 is expressed in erythrocytes. Defects in the AMPD1 gene result in adenosine monophosphate deaminase deficiency muscle type (AMPDDM). AMPDDM is a metabolic disorder resulting in exercise-related myopathy and is characterized by exercise-induced muscle aches, cramps, and early fatigue.

REFERENCES

1. Mahnke-Zizelman, D.K., et al. 1996. Cloning, sequence and characterization of the human AMPD2 gene: evidence for transcriptional regulation by two closely spaced promoters. *Biochim. Biophys. Acta* 1308: 122-132.
2. Mahnke-Zizelman, D.K., et al. 1997. Regulation of rat AMP deaminase 3 (isoform C) by development and skeletal muscle fibre type. *Biochem. J.* 326: 521-529.
3. Mahnke-Zizelman, D.K., et al. 2001. Localization of N-terminal sequences in human AMP deaminase isoforms that influence contractile protein binding. *Biochem. Biophys. Res. Commun.* 285: 489-495.
4. Haas, A.L., et al. 2003. Expression, purification, and inhibition of *in vitro* proteolysis of human AMPD2 (isoform L) recombinant enzymes. *Protein Expr. Purif.* 27: 293-303.
5. Szydlowska, M., et al. 2004. Full-size form of human liver AMP-deaminase? *Mol. Cell. Biochem.* 266: 133-137.
6. Fischer, S., et al. 2005. Clinical significance and neuropathology of primary MADD in C34-T and G468-T mutations of the AMPD1 gene. *Clin. Neuropathol.* 24: 77-85.

CHROMOSOMAL LOCATION

Genetic locus: AMPD1 (human) mapping to 1p13.2.

PRODUCT

AMPD1 siRNA (h) 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 AMPD1 shRNA Plasmid (h): sc-78635-SH and AMPD1 shRNA (h) Lentiviral Particles: sc-78635-V as alternate gene silencing products.

For independent verification of AMPD1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-78635A, sc-78635B and sc-78635C.

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

AMPD1 siRNA (h) is recommended for the inhibition of AMPD1 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

AMPD1 (D-7): sc-393117 is recommended as a control antibody for monitoring of AMPD1 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 AMPD1 gene expression knockdown using RT-PCR Primer: AMPD1 (h)-PR: sc-78635-PR (20 μl). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

RESEARCH USE

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

PROTOCOLS

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