SANTA CRUZ BIOTECHNOLOGY, INC.

AMPD2 siRNA (h): sc-78844

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. AMPD2 (adenosine monophosphate deaminase 2, isoform L), also known as liver-type AMP deaminase, is a member of the adenosine and AMP deaminases family and is involved in the degradation of adenylic acid in human term placenta. Due to alternative splicing of the gene, four isoforms exist for AMPD2.

REFERENCES

1. Van den Bergh, F., et al. 1995. Characterization of human AMP deaminase 2 (AMPD2) gene expression reveals alternative transcripts encoding variable N-terminal extensions of isoform L. Biochem. J. 312: 401-410.
2. 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.
3. 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.
4. Toledo, F., et al. 1999. Initiation of DNA replication at the Chinese hamster origin oriGNAI3 relies on local sequences and/or chromatin structures, but not on transcription of the nearby GNAI3 gene. Nucleic Acids Res. 27: 1600-1608.
5. 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.
6. Haas, A.L., et al. 2003. N-terminal extensions of the human AMPD2 polypeptide influence ATP regulation of isoform L. Biochem. Biophys. Res. Commun. 305: 421-427.

CHROMOSOMAL LOCATION

Genetic locus: AMPD2 (human) mapping to 1p13.3.

PRODUCT

AMPD2 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 AMPD2 shRNA Plasmid (h): sc-78844-SH and AMPD2 shRNA (h) Lentiviral Particles: sc-78844-V as alternate gene silencing products.

For independent verification of AMPD2 (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-78844A, sc-78844B and sc-78844C.

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

AMPD2 siRNA (h) is recommended for the inhibition of AMPD2 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

AMPD2 (QQ13): sc-100504 is recommended as a control antibody for monitoring of AMPD2 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 AMPD2 gene expression knockdown using RT-PCR Primer: AMPD2 (h)-PR: sc-78844-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.