SANTA CRUZ BIOTECHNOLOGY, INC.

IVD siRNA (m): sc-62512

BACKGROUND

IVD (isovaleryl-CoA dehydrogenase, mitochondrial) is a 423 amino acid protein encoded by the human gene IVD. IVD is a mitochondrion matrix protein that belongs to the acyl-CoA dehydrogenase family. IVD is a homotetrameric flavoenzyme which catalyzes the conversion of isovaleryl-CoA to 3-methylcrotonyl-CoA. Defects of the IVD gene lead to ineffective isoforms that are the underlying cause of isovaleric acidemia. Two forms of isovaleric acidemia, possibly allelic, are recognized: the acute neonatal form, leading to massive metabolic acidosis from the first days of life and rapid death, and a chronic form in which periodic attacks of severe ketoacidosis occur with asymptomatic intervening periods. There are seven classes of mutants, each with different deletions and pathologies.

REFERENCES

1. Vockley, J., et al. 1992. The variant human isovaleryl-CoA dehydrogenase gene responsible for type II isovaleric acidemia determines an RNA splicing error, leading to the deletion of the entire second coding exon and the production of a truncated precursor protein that interacts poorly with mitochondrial import receptors. J. Biol. Chem. 267: 2494-2501.
2. Parimoo, B., et al. 1993. Structural organization of the human isovaleryl-CoA dehydrogenase gene. Genomics 15: 582-590.
3. Vockley, J., et al. 2000. Exon skipping in IVD RNA processing in isovaleric acidemia caused by point mutations in the coding region of the IVD gene. Am. J. Hum. Genet. 66: 356-367.
4. Tajima, G., et al. 2005. Establishment of a practical enzymatic assay method for determination of isovaleryl-CoA dehydrogenase activity using high-performance liquid chromatography. Clin. Chim. Acta 353: 193-199.
5. Goetzman, E.S., et al. 2006. Functional analysis of acyl-CoA dehydrogenase catalytic residue mutants using surface plasmon resonance and circular dichroism. Mol. Genet. Metab. 87: 233-242.
6. Vockley, J., et al. 2006. Isovaleric acidemia: new aspects of genetic and phenotypic heterogeneity. Am. J. Med. Genet. C Semin. Med. Genet. 142: 95-103.

CHROMOSOMAL LOCATION

Genetic locus: Ivd (mouse) mapping to 2 E5.

PRODUCT

IVD siRNA (m) 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 IVD shRNA Plasmid (m): sc-62512-SH and IVD shRNA (m) Lentiviral Particles: sc-62512-V as alternate gene silencing products.

For independent verification of IVD (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-62512A, sc-62512B and sc-62512C.

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

IVD siRNA (m) is recommended for the inhibition of IVD expression in mouse 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

IVD (A-8): sc-514240 is recommended as a control antibody for monitoring of IVD 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 IVD gene expression knockdown using RT-PCR Primer: IVD (m)-PR: sc-62512-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.