SANTA CRUZ BIOTECHNOLOGY, INC.

IDH3G siRNA (h): sc-62493

BACKGROUND

IDH3G (isocitrate dehydrogenase [NAD] subunit γ (mitochondrial), NAD^+-specific ICDH) is a 393 amino acid protein encoded by the human gene IDH3G. IDH3G belongs to the isocitrate and isopropylmalate dehydrogenases family and can bind one magnesium or manganese ion per subunit. It is usually found in the mitochondrion as a heterooligomer of subunits α, β, and γ in the apparent ratio of 2:1:1. Human NAD-dependent isocitrate dehydrogenase (IDH) is allosterically activated by ADP by lowering the Km for isocitrate. NAD-dependent isocitrate dehydrogenase is a tricarboxylic acid cycle enzyme that produces 2-oxoglutarate, an organic acid required by the glutamine synthetase/glutamate synthase cycle to assimilate ammonium.

REFERENCES

1. Kim, Y.O., et al. 1995. Characterization of a cDNA clone for human NAD^+-specific isocitrate dehydrogenase α-subunit and structural comparison with its isoenzymes from different species. Biochem. J. 308: 63-68.
2. Hong, G., et al. 1997. Molecular cloning of a highly conserved mouse and human integral membrane protein (Itm1) and genetic mapping to mouse chromosome 9. Genomics 31: 295-300.
3. Huh, T.L., et al. 1997. Assignment of the human mitochondrial NAD^+-specific isocitrate dehydrogenase α subunit (IDH3A) gene to 15q25.1→q25.2 by *in situ* hybridization. Genomics 32: 295-296.
4. Dash, D.P., et al. 2006. Fine mapping of the keratoconus with cataract locus on chromosome 15q and candidate gene analysis. Mol. Vis. 12: 499-505.
5. Soundar, S., et al. 2006. Identification of Mn^{2+}-binding aspartates from α, β, and γ subunits of human NAD-dependent isocitrate dehydrogenase. J. Biol. Chem. 281: 21073-21081.
6. Imabayashi, F., et al. 2006. Substrate-free structure of a monomeric NADP isocitrate dehydrogenase: an open conformation phylogenetic relationship of isocitrate dehydrogenase. Proteins 63: 100-112.

CHROMOSOMAL LOCATION

Genetic locus: IDH3G (human) mapping to Xq28.

PRODUCT

IDH3G 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 IDH3G shRNA Plasmid (h): sc-62493-SH and IDH3G shRNA (h) Lentiviral Particles: sc-62493-V as alternate gene silencing products.

For independent verification of IDH3G (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-62493A, sc-62493B and sc-62493C.

PROTOCOLS

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

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

IDH3G siRNA (h) is recommended for the inhibition of IDH3G 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

IDH3G (C-5): sc-365489 is recommended as a control antibody for monitoring of IDH3G 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 IDH3G gene expression knockdown using RT-PCR Primer: IDH3G (h)-PR: sc-62493-PR (20 µl, 554 bp). 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.