SANTA CRUZ BIOTECHNOLOGY, INC.

IDH1 siRNA (m): sc-60830

BACKGROUND

The Isocitrate dehydrogenase (IDHC or IDH) cytoplasmic enzyme is a homodimer of 416 residues that belongs to the isocitrate and isopropylmalate dehydrogenases family. IDHC catalyzes the third step of the citric acid cycle, which involves the oxidative decarboxylation of isocitrate, forming α-ketoglutarate and CO_2 in a two step reaction. The first step involves the oxidation of isocitrate to the intermediate oxalosuccinate, while the second step involves the production of α-ketoglutarate. During this process, either NADH or NADPH is produced along with CO_2. Ca^{2+} can bind to IDHC as a complex with isocitrate, acting as a competitive inhibitor of Mg^{2+}. The IDHC enzyme is inactivated by phosphorylation at Ser 113 and contains a clasp-like domain wherein both polypeptide chains in the dimer interlock. IDHC is expressed in a wide range of species and also in organisms that lack a complete citric acid cycle.

REFERENCES

1. Thorsness, P.E. and Koshland, D.E. 1987. Inactivation of isocitrate dehydrogenase by phosphorylation is mediated by the negative charge of the phosphate. J. Biol. Chem. 262: 10422-10425.
2. Hurley, J.H., et al. 1989. Structure of a bacterial enzyme regulated by phosphorylation, isocitrate dehydrogenase. Proc. Natl. Acad. Sci. USA 86: 8635-8639.
3. Nekrutenko, A., et al. 1999. Cytosolic isocitrate dehydrogenase in humans, mice, and voles and phylogenetic analysis of the enzyme family. J. Mol. Evol. 15: 1674-1684.
4. Geisbrecht, B.V. and Gould, S.J. 1999. The human PICD gene encodes a cytoplasmic and peroxisomal $NADP^+$-dependent isocitrate dehydrogenase. J. Biol. Chem. 274: 30527-30533.

CHROMOSOMAL LOCATION

Genetic locus: Idh1 (mouse) mapping to 1 C2.

PRODUCT

IDH1 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 IDH1 shRNA Plasmid (m): sc-60830-SH and IDH1 shRNA (m) Lentiviral Particles: sc-60830-V as alternate gene silencing products.

For independent verification of IDH1 (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-60830A, sc-60830B and sc-60830C.

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

IDH1 siRNA (m) is recommended for the inhibition of IDH1 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

IDH1 (F-3): sc-515396 is recommended as a control antibody for monitoring of IDH1 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 IDH1 gene expression knockdown using RT-PCR Primer: IDH1 (m)-PR: sc-60830-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Robbins, D., et al. 2012. Isocitrate dehydrogenase 1 is downregulated during early skin tumorigenesis which can be inhibited by overexpression of manganese superoxide dismutase. Cancer Sci. 103: 1429-1433.
2. Liu, L., et al. 2016. Malic enzyme tracers reveal hypoxia-induced switch in adipocyte NADPH pathway usage. Nat. Chem. Biol. 12: 345-352.

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.