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SANTA CRUZ BIOTECHNOLOGY, INC.

IscU1/2 siRNA (h): sc-270108

BACKGROUND

Iron-sulfur (Fe-S) clusters are cofactors that are essential for a wide variety of processes, including facilitation of electron transfer processes in oxidative phosphorylation, catalysis of enzymatic reactions in aconitase and dehydratases and maintenance of structural integrity in the DNA repair enzyme endonuclease III. In bacteria and eukaryotes, several new genes are implicated in the biogenesis of Fe-S cluster-containing proteins. IscU1 and IscU2, homologs to bacterial IscU and NifU, are iron cluster-assembly proteins. Deletion of either IscU1 or IscU2 results in increased accumulation of iron within the mitochondria, loss of activity of the [4Fe-4S] aconitase enzyme, and suppression of oxidative damage in cells lacking cytosolic copper/zinc superoxide dismutase. IscU1 and IscU2 are regulated by the iron status of the cell and localize primarily in the mitochondria. In human cells, alternative splicing of IscU pre-mRNA results in synthesis of these two proteins, which differ at the N-terminus and localize either to the cytosol (IscU1) or the mitochondria (IscU2). IscU proteins interact with IscS, a cysteine desulfurase, to sequester inorganic sulfur for Fe-S cluster assembly. IscU-IscS protein complex localizes in both mitochondria and cytosol, implying that Fe-S cluster assembly takes place in multiple subcellular compartments in mammalian cells.

REFERENCES

1. Beinert, H. and Holm, R.H. 1997 Iron-sulfur clusters: nature's modular, multipurpose structure. Science 277: 653-659.
2. Zheng, L., Cash, V.L., Flint, D.H. and Dean, D.R. 1998. Assembly of iron-clusters: identification of an iscSUA-hscBA-fdx gene cluster from *Azotobacter vinelandii*. J. Biol. Chem. 273: 13264-13272.
3. Garland, S.A., Hoff, K., Vickery, L.E. and Culotta, V.C. 1999. Saccharomyces cerevisiae ISU1 and ISU2: members of a well-conserved gene family for iron-sulfur cluster assembly. J. Mol. Biol. 294: 897-907.
4. Schilke, B., Voisine, C., Beinet, H. and Craig, E. 1999. Evidence for a conserved system for iron metabolism in the mitochondria of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 96: 10206-10211.
5. Tong, W.H. and Rouault, T. 2000. Distinct iron-sulfur cluster assembly complexes exist in the cytosol and mitochondria of human cells. EMBO J. 19: 5692-5700.

CHROMOSOMAL LOCATION

Genetic locus: ISCU (human) mapping to 12q23.3.

PRODUCT

IscU1/2 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 IscU1/2 shRNA Plasmid (h): sc-270108-SH and IscU1/2 shRNA (h) Lentiviral Particles: sc-270108-V as alternate gene silencing products.

For independent verification of IscU1/2 (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-270108A, sc-270108B and sc-270108C.

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

IscU1/2 siRNA (h) is recommended for the inhibition of IscU1/2 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

IscU1/2 (D-6): sc-373694 is recommended as a control antibody for monitoring of IscU1/2 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 IscU1/2 gene expression knockdown using RT-PCR Primer: IscU1/2 (h)-PR: sc-270108-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.