SANTA CRUZ BIOTECHNOLOGY, INC.

IRP-1 siRNA (h): sc-40713

BACKGROUND

Iron metabolism is essential for sustaining mammalian homeostasis. Iron uptake and distribution is a highly regulated process in mammalian cells that is monitored by two iron sensing proteins; iron regulatory protein-1 and -2 (IRP-1 and -2), also known as iron responsive element-binding protein-1 and -2 (IRE-BP-1 and -2) or aconitase 1 and 2. IRP-1 and IRP-2 are important soluble regulatory factors that mediate iron uptake and storage in mammalian cells. They are capable of either repressing translation or enhancing mRNA stability by associating with stem-loop motifs known as iron-responsive elements (IREs). IRPs respond to stress mediators, iron concentration and signaling factors, including nitrogen monoxide, cytokines and hydrogen peroxide.

REFERENCES

1. Rouault, T.A., et al. 1990. Cloning of the cDNA encoding an RNA regulatory protein—the human iron-responsive element-binding protein. Proc. Natl. Acad. Sci. USA 87: 7958-7962.
2. Kaptain, S., et al. 1991. A regulated RNA binding protein also possesses aconitase activity. Proc. Natl. Acad. Sci. USA 88: 10109-10113.
3. Hentze, M.W., et al. 1991. Homology between IRE-BP, a regulatory RNA-binding protein, aconitase, and isopropylmalate isomerase. Nucleic Acids Res. 19: 1739-1740.
4. Hirling, H., et al. 1992. Expression of active iron regulatory factor from a full-length human cDNA by *in vitro* transcription/translation. Nucleic Acids Res. 20: 33-39.
5. Rouault, T.A., et al. 1996. The impact of oxidative stress on eukaryotic iron metabolism. EXS 77: 183-197.

CHROMOSOMAL LOCATION

Genetic locus: ACO1 (human) mapping to 9p21.1.

PRODUCT

IRP-1 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 IRP-1 shRNA Plasmid (h): sc-40713-SH and IRP-1 shRNA (h) Lentiviral Particles: sc-40713-V as alternate gene silencing products.

For independent verification of IRP-1 (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-40713A, sc-40713B and sc-40713C.

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

IRP-1 siRNA (h) is recommended for the inhibition of IRP-1 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

IRP-1 (E-12): sc-166022 is recommended as a control antibody for monitoring of IRP-1 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 IRP-1 gene expression knockdown using RT-PCR Primer: IRP-1 (h)-PR: sc-40713-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. Roy, A. and Kolattukudy, P.E. 2012. Monocyte chemotactic protein-induced protein (MCPIP) promotes inflammatory angiogenesis via sequential induction of oxidative stress, endoplasmic reticulum stress and autophagy. Cell. Signal. 24: 2123-2131.
2. Jiang, X., et al. 2014. Hyperinsulinemia induces hepatic iron overload by increasing liver TFR1 via the PI3K/IRP-2 pathway. J. Mol. Endocrinol. 53: 381-92.

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.