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HFE siRNA (h): sc-43832

BACKGROUND

The features of hemochromatosis include cirrhosis of the liver, diabetes, hypermelanotic pigmentation of the skin and heart failure. Since hemochromatosis is a relatively easily treated disorder if diagnosed, this is a form of preventable cancer. The HFE protein, which is defective in hereditary hemochromatosis, normally is expressed in crypt enterocytes of the duodenum where it has a unique, predominantly intracellular localization. In placenta, the HFE protein co-localizes with and forms a stable association with the transferrin receptor (TFR), providing a link between the HFE protein and iron transport. Immunocytochemistry shows that the HFE protein and TFR both are expressed in the crypt enterocytes. Western blots show that, as is the case in human placenta, the HFE protein in crypt enterocytes is physically associated with the TFR and with β 2-microglobulin. It is proposed that HFE has two mutually exclusive activities in cells: inhibition of uptake or inhibition of release of iron and that the balance between serum transferrin saturation and serum transferrin-receptor concentrations determines which of these functions predominates. The gene which encodes HFE maps to human chromosome 6p22.2.

REFERENCES

1. Cragg, S.J., Drysdale, J. and Worwood, M. 1985. Genes for the "H" sub-unit of human ferritin are present on a number of human chromosomes. *Hum. Genet.* 71: 108-112.
2. McGill, J.R., Naylor, S.L., Sakaguchi, A.Y., Moore, C.M., Boyd, D., Barrett, K.J., Shows, T.B. and Drysdale, J.W. 1987. Human ferritin H and L sequences lie on ten different chromosomes. *Hum. Genet.* 76: 66-72.
3. Waheed, A., Parkkila, S., Saarnio, J., Fleming, R.E., Zhou, X.Y., Tomatsu, S., Britton, R.S., Bacon, B.R. and Sly, W.S. 1999. Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. *Proc. Natl. Acad. Sci. USA* 96: 1579-1584.
4. Townsend, A. and Drakesmith, H. 2002. Role of HFE in iron metabolism, hereditary haemochromatosis, anaemia of chronic disease, and secondary iron overload. *Lancet* 359: 786-790.

CHROMOSOMAL LOCATION

Genetic locus: HFE (human) mapping to 6p22.2.

PRODUCT

HFE 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HFE shRNA Plasmid (h): sc-43832-SH and HFE shRNA (h) Lentiviral Particles: sc-43832-V as alternate gene silencing products.

For independent verification of HFE (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-43832A, sc-43832B and sc-43832C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

HFE siRNA (h) is recommended for the inhibition of HFE 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 μ M in 66 μ l. 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

HFE (C-12): sc-514405 is recommended as a control antibody for monitoring of HFE 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 HFE gene expression knockdown using RT-PCR Primer: HFE (h)-PR: sc-43832-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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