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HFE shRNA (h) Lentiviral Particles: sc-43832-V

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

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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.
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CHROMOSOMAL LOCATION

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

PRODUCT

HFE shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see HFE siRNA (h): sc-43832 and HFE shRNA Plasmid (h): sc-43832-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

HFE shRNA (h) Lentiviral Particles is recommended for the inhibition of HFE expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

HFE (W-24): sc-133654 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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