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HLTF shRNA (h) Lentiviral Particles: sc-45943-V

BACKGROUND

The protein encoded by the HLTF gene is a member of the SWI/SNF family of proteins. Members of this family have helicase and ATPase activities. They are thought to regulate transcription of certain genes by altering the chromatin structure around those genes. The HLTF encoded protein contains a RING finger DNA-binding motif. Two transcript variants encoding the same protein have been found for this gene. However, use of an alternative translation start site produces an isoform which is truncated at the N-terminus as compared to the full-length protein. Transcriptional inactivation of HLTF by aberrant DNA methylation and histone deacetylation may be involved in stomach carcinogenesis through down-regulation of HLTF expression.

REFERENCES

1. Mansharamani, M., et al. 2001. Cloning and characterization of an atypical type IV P-type ATPase that binds to the RING motif of RUSH transcription factors. *J. Biol. Chem.* 276: 3641-3649.
2. Moinova, H.R., et al. 2002. HLTF gene silencing in human colon cancer. *Proc. Natl. Acad. Sci. USA* 99: 4562-4567.
3. Hamai, Y., et al. 2003. DNA hypermethylation and histone hypoacetylation of the HLTF gene are associated with reduced expression in gastric carcinoma. *Cancer Sci.* 94: 692-698.
4. Leung, W.K., et al. 2003. Inactivation of helicase-like transcription factor by promoter hypermethylation in human gastric cancer. *Mol. Carcinog.* 37: 91-97.
5. SWISS-PROT/TrEMBL (NP_003062). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: HRLF (human) mapping to 3q24.

PRODUCT

HLTF 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 HLTF siRNA (h): sc-45943 and HLTF shRNA Plasmid (h): sc-45943-SH as alternate gene silencing products.

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.

APPLICATIONS

HLTF shRNA (h) Lentiviral Particles is recommended for the inhibition of HLTF 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

HLTF (G-6): sc-398357 is recommended as a control antibody for monitoring of HLTF 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HLTF gene expression knockdown using RT-PCR Primer: HLTF (h)-PR: sc-45943-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

PROTOCOLS

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