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HDAC8 shRNA (h2) Lentiviral Particles: sc-44305-V

BACKGROUND

In the intact cell, DNA closely associates with histones and other nuclear proteins to form chromatin. The remodeling of chromatin is believed to be a critical component of transcriptional regulation and a major source of this remodeling is brought about by the acetylation of nucleosomal histones. Acetylation of lysine residues in the amino-terminal tail domain of histone results in an allosteric change in the nucleosomal conformation and an increased accessibility to transcription factors by DNA. Conversely, the deacetylation of histones is associated with transcriptional silencing. Several mammalian proteins have been identified as nuclear histone acetylases, including GCN5, PCAF (p300/CBP-associated factor), p300/CBP, HAT1 and the TFIID subunit TAF II p250. Mammalian HDAC8, isolated from human kidney, is a histone deacetylase that shares homology to other HDACs but has different tissue distribution. HDAC8 is localized to the nucleus and plays a role in the development of a broad range of tissues and in the etiology of cancer.

REFERENCES

1. Lee, D.Y., et al. 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell* 72: 73-82.
2. Braunstein, M., et al. 1993. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev.* 7: 592-604.
3. Bauer, W.R., et al. 1994. Nucleosome structural changes due to acetylation. *J. Mol. Biol.* 236: 685-690.
4. Utey, R.T., et al. 1998. Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. *Nature* 394: 498-502.
5. Verreault, A., et al. 1998. Nucleosomal DNA regulates the core-histone-binding subunit of the human HAT1 acetyltransferase. *Curr. Biol.* 8: 96-108.
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CHROMOSOMAL LOCATION

Genetic locus: HDAC8 (human) mapping to Xq13.1.

PRODUCT

HDAC8 shRNA (h2) 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 HDAC8 siRNA (h2): sc-44305 and HDAC8 shRNA Plasmid (h2): sc-44305-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

HDAC8 shRNA (h2) Lentiviral Particles is recommended for the inhibition of HDAC8 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

HDAC8 (B-4): sc-365620 is recommended as a control antibody for monitoring of HDAC8 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 HDAC8 gene expression knockdown using RT-PCR Primer: HDAC8 (h2)-PR: sc-44305-PR (20 μ l, 593 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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