SANTA CRUZ BIOTECHNOLOGY, INC.

HDAC8 siRNA (h2): sc-44305

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. Utley, 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.
6. Hu, E., et al. 2000. Cloning and characterization of a novel human class I histone deacetylas that functions as a transcription repressor. J. Biol. Chem. 275: 15254-15264.
7. Waltregny, D., et al. 2004. Expression of histone deacetylase 8, a class I histone deacetylase, is restricted to cells showing smooth muscle differentiation in normal human tissues. Am. J. Pathol. 165: 553-564.
8. Somoza, J.R., et al. 2005. Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. Structure 12: 1325-1334.

CHROMOSOMAL LOCATION

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

PRODUCT

HDAC8 siRNA (h2) 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 HDAC8 shRNA Plasmid (h2): sc-44305-SH and HDAC8 shRNA (h2) Lentiviral Particles: sc-44305-V as alternate gene silencing products.

For independent verification of HDAC8 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44305A, sc-44305B and sc-44305C.

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

HDAC8 siRNA (h2) is recommended for the inhibition of HDAC8 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

HDAC8 (E-5): sc-17778 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 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 HDAC8 gene expression knockdown using RT-PCR Primer: HDAC8 (h2)-PR: sc-44305-PR (20 µl, 593 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.