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CHD8 siRNA (m): sc-142322

BACKGROUND

CHD8 (chromodomain helicase DNA binding protein 8), also known as HELSNF1 or KIAA1564, is a 2,581 amino acid protein that localizes to the nucleus and contains one helicase C-terminal domain, one helicase ATP-binding domain and two chromo domains. Functioning as a DNA helicase, CHD8 acts as a transcriptional repressor that remodels chromatin structure and represses p53-mediated apoptosis, specifically by recruiting Histone H1 to target genes and preventing p53 transactivation activity. CHD8 exists as multiple alternatively spliced isoforms and is subject to post-translational sumoylation. The gene encoding CHD8 maps to human chromosome 14, chromosome 14, which houses over 700 genes and comprises nearly 3.5% of the human genome. Chromosome 14 encodes the presenilin 1 (PSEN1) gene, which is one of the three key genes associated with the development of Alzheimer's disease (AD). The SERPINA1 gene is also located on chromosome 14 and, when defective, leads to the genetic disorder α 1-antitrypsin deficiency, which is characterized by severe lung complications and liver dysfunction.

REFERENCES

1. Sakamoto, I., et al. 2000. A novel β -catenin-binding protein inhibits β -catenin-dependent TCF activation and axis formation. *J. Biol. Chem.* 275: 32871-32878.
2. Kobayashi, M., et al. 2002. Nuclear localization of Duplin, a β -catenin-binding protein, is essential for its inhibitory activity on the Wnt signaling pathway. *J. Biol. Chem.* 277: 5816-5822.
3. Nishiyama, M., et al. 2004. Early embryonic death in mice lacking the β -catenin-binding protein Duplin. *Mol. Cell. Biol.* 24: 8386-8394.
4. Ishihara, K., et al. 2006. CTCF-dependent chromatin insulator is linked to epigenetic remodeling. *Mol. Cell* 23: 733-742.

CHROMOSOMAL LOCATION

Genetic locus: Chd8 (mouse) mapping to 14 C2.

PRODUCT

CHD8 siRNA (m) 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 CHD8 shRNA Plasmid (m): sc-142322-SH and CHD8 shRNA (m) Lentiviral Particles: sc-142322-V as alternate gene silencing products.

For independent verification of CHD8 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-142322A, sc-142322B and sc-142322C.

RESEARCH USE

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

PROTOCOLS

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

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

CHD8 siRNA (m) is recommended for the inhibition of CHD8 expression in mouse 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

CHD8 (C-16): sc-104835 is recommended as a control antibody for monitoring of CHD8 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CHD8 gene expression knockdown using RT-PCR Primer: CHD8 (m)-PR: sc-142322-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.