

Pr-Set7 siRNA (m): sc-155946

BACKGROUND

The methylation of histones plays a pivotal role in the regulation of chromatin structure and gene expression. Histone methylation can occur on Arg or Lys residues, with an exquisite site selectivity for Lys methylation at specific positions in the N-termini of Histones H3 and H4. Pr-Set7, also referred to as SET8, is a nucleosome-specific monomethylase that specifically methylates H4 at Lys 20, a mark of constitutive and facultative heterochromatin. Pr-Set7 is a single subunit enzyme and prefers nucleosomal substrates. It functions to regulate cell-cycle-dependent transcriptional silencing and mitotic regulation in metazoans. The amino acid sequence RHRKVLRDN (17-25) is required for the SET domain of Pr-Set7 to function and, thus, for multiplicity of methylation of Lys 20 of H4 to occur. The methylation mark is very stable and is maintained even in the absence of Pr-Set7.

REFERENCES

1. Fang, J., et al. 2002. Purification and functional characterization of SET8, a nucleosomal Histone H4-Lysine 20-specific methyltransferase. *Curr. Biol.* 12: 1086-1099.
2. Rice, J.C., et al. 2002. Mitotic-specific methylation of Histone H4 Lys 20 follows increased Pr-Set7 expression and its localization to mitotic chromosomes. *Genes Dev.* 16: 2225-2230.
3. Nishioka, K., et al. 2002. Pr-Set7 is a nucleosome-specific methyltransferase that modifies Lysine 20 of Histone H4 and is associated with silent chromatin. *Mol. Cell* 9: 1201-1213.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607240. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. Karachentsev, D., et al. 2005. Pr-Set7-dependent methylation of Histone H4 Lys 20 functions in repression of gene expression and is essential for mitosis. *Genes Dev.* 19: 431-435.
6. Xiao, B., et al. 2005. Specificity and mechanism of the histone methyltransferase Pr-Set7. *Genes Dev.* 19: 1444-1454.
7. Couture, J.F., et al. 2005. Structural and functional analysis of SET8, a Histone H4 Lys 20 methyltransferase. *Genes Dev.* 19: 1455-1465.

CHROMOSOMAL LOCATION

Genetic locus: Setd8 (mouse) mapping to 5 F.

PRODUCT

Pr-Set7 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 Pr-Set7 shRNA Plasmid (m): sc-155946-SH and Pr-Set7 shRNA (m) Lentiviral Particles: sc-155946-V as alternate gene silencing products.

For independent verification of Pr-Set7 (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-155946A, sc-155946B and sc-155946C.

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

Pr-Set7 siRNA (m) is recommended for the inhibition of Pr-Set7 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

Pr-Set7 (D-11): sc-377034 is recommended as a control antibody for monitoring of Pr-Set7 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Pr-Set7 gene expression knockdown using RT-PCR Primer: Pr-Set7 (m)-PR: sc-155946-PR (20 μ l). 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.