

HiNF-P siRNA (m): sc-60791

BACKGROUND

HiNF-P is a critical transcription factor which is necessary for E2F-independent activation of the Histone H4 multigene family. HiNF-P associates with conserved H4 cell cycle regulatory sequences *in vivo*. Antisense inhibition of HiNF-P reduces endogenous Histone H4 gene expression. HiNF-P utilizes NPAT/p220, a substrate of the cyclin E/cyclin-dependent kinase 2 (CDK2) kinase complex, as a crucial coactivator to amplify Histone H4 gene transcription. The biological role of HiNF-P is reflected by impeded cell cycle progression into S phase upon antisense-mediated reduction of HiNF-P levels. Research indicates that HiNF-P is the key link in a linear signaling pathway that is initiated with the growth factor-dependent induction of cyclin E/CDK2 kinase activity at the restriction point and culminates in the activation of Histone H4 genes through HiNF-P at the G₁/S phase transition.

REFERENCES

1. van Wijnen, A.J., et al. 1991. Transcriptional element H4-site II of cell cycle regulated human H4 Histone genes is a multipartite protein/DNA interaction site for factors HiNF-D, HiNF-M, and HiNF-P: involvement of phosphorylation. *J. Cell. Biochem.* 46: 174-189.
2. van den Ent, F.M., et al. 1993. Concerted control of multiple histone promoter factors during cell density inhibition of proliferation in osteosarcoma cells: reciprocal regulation of cell cycle-controlled and bone-related genes. *Cancer Res.* 53: 2399-2409.
3. Aziz, F., et al. 1998. HiNF-D (CDP-cut/CDC2 cell cycle activation of human Histone H4 gene transcription at the G₁/S phase transition. *J. Cell. Physiol.* 177: 453-464.
4. Aziz, F., et al. 1998. The integrated activities of IRF-2 (HiNF-M), CDP/cut (HiNF-D) and H4TF-2 (HiNF-P) regulate transcription of a cell cycle controlled human Histone H4 gene: mechanistic differences between distinct H4 genes. *Mol. Biol. Rep.* 25: 1-12.
5. Hovhannisyann, H., et al. 2003. Maintenance of open chromatin and selective genomic occupancy at the cell cycle-regulated Histone H4 promoter during differentiation of HL-60 promyelocytic leukemia cells. *Mol. Cell. Biol.* 23: 1460-1469.

CHROMOSOMAL LOCATION

Genetic locus: Hinfp (mouse) mapping to 9 A5.2.

PRODUCT

HiNF-P 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 HiNF-P shRNA Plasmid (m): sc-60791-SH and HiNF-P shRNA (m) Lentiviral Particles: sc-60791-V as alternate gene silencing products.

For independent verification of HiNF-P (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-60791A, sc-60791B and sc-60791C.

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

HiNF-P siRNA (m) is recommended for the inhibition of HiNF-P 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

HiNF-P (C-5): sc-373855 is recommended as a control antibody for monitoring of HiNF-P 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 HiNF-P gene expression knockdown using RT-PCR Primer: HiNF-P (m)-PR: sc-60791-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.