SANTA CRUZ BIOTECHNOLOGY, INC.

HP1β siRNA (h): sc-35587

BACKGROUND

Chromatin assembly factor-1 (CAF-1) is a multisubunit protein complex that comprises three polypeptide subunits known as p150, p60, and p48. CAF-1 is a nucleosome assembly factor that deposits newly synthesized and acetylated Histones H3/H4 into nascent chromatin during DNA replication. The p150 subunit of CAF-1 also supports the maintenance of heterochromatin, which requires the synthesis of both new Histones and heterochromatin proteins and their orderly assembly during DNA replication. Heterochromatin is characterized as densely coiled chromatin that generally replicates late during S phase, has a low gene density and contains large blocks of repetitive DNA that is relatively inaccessible to DNA-modifying reagents. In late S phase, p150 directly associates with heterochromatin associated proteins 1 (HP1α, HP1β and HP1γ). As cells prepare for mitosis, CAF-1 p150 and some HP1 progressively dissociate from heterochromatin, coinciding with the phosphorylation of Histone H3. The HP1 proteins reassociate with chromatin at the end of mitosis, as Histone H3 is dephosphorylated.

REFERENCES

1. Smith, S., et al. 1989. Purification and characterization of CAF-I, a human cell factor required for chromatin assembly during DNA replication *in vitro*. Cell 58: 15-25.
2. Kaufman, P.D., et al. 1995. The p150 and p60 subunits of chromatin assembly factor I: a molecular link between newly synthesized histones and DNA replication. Cell 81: 1105-1114.
3. Verreault, A., et al. 1996. Nucleosome assembly by a complex of CAF-1 and acetylated Histones H3/H4. Cell 87: 95-104.
4. Minc, E., et al. 1999. Localization and phosphorylation of HP1 proteins during the cell cycle in mammalian cells. Chromosoma 108: 220-234.
5. Taddei, A., et al. 1999. Duplication and maintenance of heterochromatin domains. J. Cell Biol. 147: 1153-1166.
6. Murzina, N., et al. 1999. Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins. Mol. Cell 4: 529-540.

CHROMOSOMAL LOCATION

Genetic locus: CBX1 (human) mapping to 17q21.32.

PRODUCT

HP1β siRNA (h) 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 HP1β shRNA Plasmid (h): sc-35587-SH and HP1β shRNA (h) Lentiviral Particles: sc-35587-V as alternate gene silencing products.

For independent verification of HP1β (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35587A, sc-35587B and sc-35587C.

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

HP1β siRNA (h) is recommended for the inhibition of HP1β 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

HP1β (4D7B8): sc-517288 is recommended as a control antibody for monitoring of HP1β 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 HP1β gene expression knockdown using RT-PCR Primer: HP1β (h)-PR: sc-35587-PR (20 µl, 427 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.