SANTA CRUZ BIOTECHNOLOGY, INC.

Hic-5 siRNA (m): sc-37686

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The Power to Question

BACKGROUND

In addition to paxillin, zysin, LPP, Ajuba and TRIP6, hydrogen-peroxide inducible clone 5 (Hic-5) is a member of the LIM family. Hic-5 contains four LIM motifs and seven zinc finger domains. In the cell, Hic-5 localizes to the nuclear matrix and focal adhesion complexes where the LIM domains mediate the interactions of Hic-5 with focal adhesions. Known also as transforming factor β1 induced transcript 1, Hic-5 shares extensive homology with the structural protein paxillin, which is involved in the regulation of focal adhesion dynamics. Hic-5 inhibits integrin-mediated cell spreading on Fibronectin by out competing paxillin for focal adhesion kinase and thereby preventing downstream signal transduction. Increased expression of Hic-5 leads to cellular senescence in developing fibroblasts. During myogenesis, expression of Hic-5 blocks differentiation and induces apoptosis of developing myoblasts. The gene encoding human Hic-5 maps to chromosome 16p11.2.

REFERENCES

1. Shibanuma, M., et al. 1993. Cloning from a mouse osteoblastic cell line of a set of transforming growth factor β1-regulated genes, one of which seems to encode a follistatin-related polypeptide. Eur. J. Biochem. 217: 13-19.
2. Shibanuma, M., et al. 1994. Characterization of the TGFβ1-inducible Hic-5 gene that encodes a putative novel zinc finger protein and its possible involvement in cellular senescence. J. Biol. Chem. 269: 26767-26774.
3. Shibanuma, M., et al. 1997. Induction of senescence-like phenotypes by forced expression of Hic-5, which encodes a novel LIM motif protein, in immortalized human fibroblasts. Mol. Cell. Biol. 17: 1224-1235.
4. Matsuya, M., et al. 1998. Cell adhesion kinase forms a complex with a new member, Hic-5, of proteins localized at focal adhesions. J. Biol. Chem. 273: 1003-1014.
5. Fujita, H., et al. 1998. Interaction of Hic-5, a senescence-related protein, with focal adhesion kinase. J. Biol. Chem. 273: 26516-26521.
6. Thomas, S.M., et al. 1999. Characterization of a focal adhesion protein, Hic-5, that shares extensive homology with paxillin. J. Cell Sci. 112: 181-190.

CHROMOSOMAL LOCATION

Genetic locus: Tgfb1i1 (mouse) mapping to 7 F3.

PRODUCT

Hic-5 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Hic-5 shRNA Plasmid (m): sc-37686-SH and Hic-5 shRNA (m) Lentiviral Particles: sc-37686-V as alternate gene silencing products.

For independent verification of Hic-5 (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-37686A, sc-37686B and sc-37686C.

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

Hic-5 siRNA (m) is recommended for the inhibition of Hic-5 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 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

Hic-5 (C-6): sc-271353 is recommended as a control antibody for monitoring of Hic-5 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 Hic-5 gene expression knockdown using RT-PCR Primer: Hic-5 (m)-PR: sc-37686-PR (20 µl, 543 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.