SANTA CRUZ BIOTECHNOLOGY, INC.

FHL-5 siRNA (h): sc-95151

BACKGROUND

The four-and-a-half-LIM domain (FHL) proteins include FHL-1 (SLIM1), FHL-2 (SLIM3), FHL-3 (SLIM2), FHL-4 and FHL-5. The signature "half-domain", a single zinc finger domain located in the N-terminal region, differentiates FHLs from other LIM-only proteins, which have numbers of zinc fingers. Specific combinations of FHL proteins elicit selective activation of both CREB and CREM. FHL-5, also known as ACT (activator of CREM in testis), is a testis-specific protein that interacts with CREM (a transcription factor required for spermatid differentiation) via its third LIM domain and can stimulate CREM activity independently of phosphorylation. This suggests that FHL-5 may participate in the regulation of spermatogenesis by acting as a transcriptional coactivator of CREM. During spermatid elongation, FHL-5 is translocated from the nucleus to the cytoplasm by the kinesin motor protein KIF17 thus silencing CREM activity.

REFERENCES

1. Fimia, G.M., et al. 1998. Mechanisms of activation by CREB and CREM: phosphorylation, CBP, and a novel coactivator, ACT. Cold Spring Harb. Symp. Quant. Biol. 63: 631-642.
2. Morgan, M.J. and Whawell, S.A. 2000. The structure of the human LIM protein ACT gene and its expression in tumor cell lines. Biochem. Biophys. Res. Commun. 273: 776-783.
3. Palermo, I., et al. 2001. Cloning and expression of activator of CREM in testis in human testicular tissue. Biochem. Biophys. Res. Commun. 283: 406-411.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605126. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Mistry, A.C., et al. 2004. FHL5, a novel Actin-binding protein, is highly expressed in eel gill pillar cells and responds to wall tension. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287: R1141-R1154.
6. Kotaja, N., et al. 2004. Abnormal sperm in mice with targeted deletion of the act (activator of cAMP-responsive element modulator in testis) gene. Proc. Natl. Acad. Sci. USA 101: 10620-10625.

CHROMOSOMAL LOCATION

Genetic locus: FHL5 (human) mapping to 6q16.1.

PRODUCT

FHL-5 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 FHL-5 shRNA Plasmid (h): sc-95151-SH and FHL-5 shRNA (h) Lentiviral Particles: sc-95151-V as alternate gene silencing products.

For independent verification of FHL-5 (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-95151A, sc-95151B and sc-95151C.

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

FHL-5 siRNA (h) is recommended for the inhibition of FHL-5 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

FHL-5 (F-26): sc-101045 is recommended as a control antibody for monitoring of FHL-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 FHL-5 gene expression knockdown using RT-PCR Primer: FHL-5 (h)-PR: sc-95151-PR (20 µl). 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.