

FHL-4 siRNA (m): sc-35370

BACKGROUND

The four-and-a-half-LIM domain (FHL) proteins include FHL-1 (SLIM1), FHL-2 (SLIM3), FHL-3 (SLIM2) and FHL-4. 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. Skeletal and cardiac muscle express FHL-1 in high levels as compared to the low level of expression in smooth muscle of the colon, small intestine and prostate. FHL-1 localizes to the cytosol of myoblasts, myotubes, and differentiated myocytes. FHL-2 is also located in cardiac and skeletal muscle, as well as in placenta and ovary tissues. FHL-3 is found in skeletal muscle, but absent in cardiac muscle. FHL-4 is expressed exclusively by the seminiferous epithelium of the testis, which suggests that FHL-4 is involved in spermatogenesis. The genetic loci for FHLs vary considerably despite similar amino acid sequences among the FHL group.

REFERENCES

1. Morgan, M.J. and Madgwick, A.J. 1996. SLIM defines a novel family of LIM-proteins expressed in skeletal muscle. *Biochem. Biophys. Res. Commun.* 225: 632-638.
2. Chan, K.K., et al. 1998. Molecular cloning and characterization of FHL-2, a novel LIM domain protein preferentially expressed in human heart. *Gene* 210: 345-350.
3. Lee, S.M., et al. 1998. Chromosomal mapping, tissue distribution and cDNA sequence of four-and-a-half LIM domain protein 1 (FHL-1). *Gene* 216: 163-170.
4. Lee, S.M., et al. 1998. Chromosomal mapping of a skeletal muscle specific LIM-only protein FHL-3 to the distal end of the short arm of human chromosome 1. *Somat. Cell Mol. Genet.* 24: 197-202.
5. Morgan, M.J. and Madgwick, A.J. 1999. The LIM proteins FHL-1 and FHL-3 are expressed differently in skeletal muscle. *Biochem. Biophys. Res. Commun.* 255: 245-250.
6. Morgan, M.J. and Madgwick, A.J. 1999. The fourth member of the FHL family of LIM proteins is expressed exclusively in the testis. *Biochem. Biophys. Res. Commun.* 255: 251-255.

CHROMOSOMAL LOCATION

Genetic locus: Fhl4 (mouse) mapping to 10 C1.

PRODUCT

FHL-4 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 FHL-4 shRNA Plasmid (m): sc-35370-SH and FHL-4 shRNA (m) Lentiviral Particles: sc-35370-V as alternate gene silencing products.

For independent verification of FHL-4 (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-35370A, sc-35370B and sc-35370C.

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

FHL-4 siRNA (m) is recommended for the inhibition of FHL-4 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

FHL-4 (D-10): sc-365443 is recommended as a control antibody for monitoring of FHL-4 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 FHL-4 gene expression knockdown using RT-PCR Primer: FHL-4 (m)-PR: sc-35370-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.