SANTA CRUZ BIOTECHNOLOGY, INC.

Cypher siRNA (h): sc-77078

BACKGROUND

Cypher, also known as LDB3 (LIM domain binding 3), ZASP (Z-band alternatively spliced PDZ-motif protein), ORACLE, PDLIM6 (PDZ and LIM domain 6), ldb3z1 or ldb3z4, is a 727 amino acid protein that localizes to the perinuclear region of the cytoplasm and contains three LIM zinc-binding domains. Expressed primarily in skeletal muscle and at lower levels in brain, placenta and heart, Cypher is thought to function as an adaptor protein that, via its LIM domains, couples PKC-mediated signaling in striated muscle to the cytoskeleton. Defects in the gene encoding Cypher are associated with dilated cardiomyopathy 1C (CMD1C), dilated cardiomyopathy with left ventricular non-compaction and ZASP-related myofibrillar myopathy (MFM), all three of which are characterized by defects in cardiac muscle form and/or function. Six isoforms of Cypher exist due to alternative splicing events.

REFERENCES

1. Zhou, Q., et al. 1999. Cypher, a striated muscle-restricted PDZ and LIM domain-containing protein, binds to α-actinin-2 and protein kinase C. J. Biol. Chem. 274: 19807-19813.
2. Passier, R., et al. 2000. Oracle, a novel PDZ-LIM domain protein expressed in heart and skeletal muscle. Mech. Dev. 92: 277-284.
3. Arimura, T., et al. 2004. A Cypher/ZASP mutation associated with dilated cardiomyopathy alters the binding affinity to protein kinase C. J. Biol. Chem. 279: 6746-6752.
4. van der Meer, D.L., et al. 2006. Zebrafish Cypher is important for somite formation and heart development. Dev. Biol. 299: 356-372.
5. Klaavuniemi, T. and Ylänne, J. 2006. Zasp/Cypher internal ZM-motif containing fragments are sufficient to co-localize with α-actinin—analysis of patient mutations. Exp. Cell Res. 312: 1299-1311.
6. Xing, Y., et al. 2006. Genetic analysis in patients with left ventricular noncompaction and evidence for genetic heterogeneity. Mol. Genet. Metab. 88: 71-77.
7. Marziliano, N., et al. 2007. Barth syndrome associated with compound hemizygosity and heterozygosity of the TAZ and LDB3 genes. Am. J. Med. Genet. A 143A: 907-915.

CHROMOSOMAL LOCATION

Genetic locus: LDB3 (human) mapping to 10q23.2.

PRODUCT

Cypher siRNA (h) is a target-specific 19-25 nt siRNA 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 Cypher shRNA Plasmid (h): sc-77078-SH and Cypher shRNA (h) Lentiviral Particles: sc-77078-V as alternate gene silencing products.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

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

Cypher (E-8): sc-374359 is recommended as a control antibody for monitoring of Cypher 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 Cypher gene expression knockdown using RT-PCR Primer: Cypher (h)-PR: sc-77078-PR (20 µl, 394 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.