SANTA CRUZ BIOTECHNOLOGY, INC.

Hugl-1 siRNA (m): sc-60819

BACKGROUND

Hugl-1 is a cortical cytoskeleton protein involved in the regulation of mitotic spindle orientation, differentiation, proliferation and tissue organization of neuroepithelial cells. It localizes to the cytoplasm and is found in a complex involved in maintaining cell polarity and epithelial integrity. Hugl-1 is associated with nonmuscle Myosin II heavy chain and interacts with PRKCI/aPKC, PARD6B/Par-6, PARD6A and STX4A. The Hugl-1 protein is expressed in kidney, brain and muscle. Expression of Hugl-1 increases cell adhesion and decreases cell migration. Hugl-1 functions as a tumor suppressor in humans, and loss of Hugl-1 expression contributes to colorectal caner and melanoma progression. LLGL1, the gene encoding for Hugl-1, has significant homology to the *Drosophila* tumor suppressor gene, l(2)gl, which encodes the protein Lgl. Like Hugl-1, Lgl is also a cortical cytoskeleton protein involved in maintaining cell polarity and epithelial integrity.

REFERENCES

1. Strand, D., et al. 1995. A human homologue of the *Drosophila* tumour suppressor gene l(2)gl maps to 17p11.2-12 and codes for a cytoskeletal protein that associates with nonmuscle Myosin II heavy chain. Oncogene 11: 291-301.
2. Koyama, K., et al. 1996. The human homologue of the murine Llglh gene (LLGL) maps within the Smith-Magenis syndrome region in 17p11.2. Cytogenet. Cell Genet. 72: 78-82.
3. Yamanaka, T., et al. 2003. Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. Curr. Biol. 13: 734-743.
4. Grifoni, D., et al. 2004. The human protein Hugl-1 substitutes for *Drosophila* lethal giant larvae tumour suppressor function *in vivo*. Oncogene 23: 8688-8694.
5. Schimanski, C.C., et al. 2005. Reduced expression of Hugl-1, the human homologue of *Drosophila* tumour suppressor gene lgl, contributes to progression of colorectal cancer. Oncogene 24: 3100-3109.
6. Huber, M.A., et al. 2005. Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr. Opin. Cell Biol. 17: 548-558.

CHROMOSOMAL LOCATION

Genetic locus: Llgl1 (mouse) mapping to 11 B2.

PRODUCT

Hugl-1 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 Hugl-1 shRNA Plasmid (m): sc-60819-SH and Hugl-1 shRNA (m) Lentiviral Particles: sc-60819-V as alternate gene silencing products.

For independent verification of Hugl-1 (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-60819A, sc-60819B and sc-60819C.

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

Hugl-1 siRNA (m) is recommended for the inhibition of Hugl-1 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

Hugl-1 (B-6): sc-136993 is recommended as a control antibody for monitoring of Hugl-1 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 Hugl-1 gene expression knockdown using RT-PCR Primer: Hugl-1 (m)-PR: sc-60819-PR (20 µl, 567 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.