SANTA CRUZ BIOTECHNOLOGY, INC.

Hugl-1 siRNA (h): sc-60818

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/αPKC, 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 cancer 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 αPKC 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.

CHROMOSOMAL LOCATION

Genetic locus: LLGL1 (human) mapping to 17p11.2.

PRODUCT

Hugl-1 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 Hugl-1 shRNA Plasmid (h): sc-60818-SH and Hugl-1 shRNA (h) Lentiviral Particles: sc-60818-V as alternate gene silencing products.

For independent verification of Hugl-1 (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-60818A, sc-60818B and sc-60818C.

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.

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 (h) is recommended for the inhibition of Hugl-1 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

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 (h)-PR: sc-60818-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.