SANTA CRUZ BIOTECHNOLOGY, INC.

PLEKHG5 siRNA (h): sc-88767

BACKGROUND

PLEKHG5 (Pleckstrin homology domain-containing family G member 5), also known as GEF720 (guanine nucleotide exchange factor 720) or DSMA4, is predominantly expressed in the peripheral nervous system and brain. PLEKHG5 localizes to the cytoplasm, however when cells are stimulated, PLEKHG5 is found near the perinuclear regions. PLEKHG5 contains the highly-conserved DH-PH module which is considered to be the signature motif of the Dbl family of guanine nucleotide exchange factors (GEFs). PLEKHG5 activates the NFκB signaling pathway and may be involved in the control of neuronal cell differentiation. It has been suggested that mutations in the PLEKHG5 gene may lead to autosomal recessive distal spinal muscular atrophy (DSMA). Four isoforms of PLEKHG5 exists due to alternative splicing events.

REFERENCES

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2. Fuentes, E.J., et al. 2003. Critical role of the Pleckstrin homology domain in Dbs signaling and growth regulation. J. Biol. Chem. 278: 21188-21196.
3. Joseph, R.E. and Norris, F.A. 2005. Substrate specificity and recognition is conferred by the Pleckstrin homology domain of the Dbl family guanine nucleotide exchange factor P-Rex2. J. Biol. Chem. 280: 27508-27512.
4. Baumeister, M.A., et al. 2006. The Dbs PH domain contributes independently to membrane targeting and regulation of guanine nucleotide-exchange activity. Biochem. J. 400: 563-572.
5. Maystadt, I., et al. 2007. The nuclear factor κB-activator gene PLEKHG5 is mutated in a form of autosomal recessive lower motor neuron disease with childhood onset. Am. J. Hum. Genet. 81: 67-76.
6. Online Mendelian Inheritance in Man, OMIM™. 2007. Johns Hopkins University, Baltimore, MD. MIM Number: 611101. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
7. Rapley, J., et al. 2008. Crucial structural role for the PH and C1 domains of the Vav1 exchange factor. EMBO Rep. 9: 655-661.

CHROMOSOMAL LOCATION

Genetic locus: PLEKHG5 (human) mapping to 1p36.31.

PRODUCT

PLEKHG5 siRNA (h) is a pool of 2 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 PLEKHG5 shRNA Plasmid (h): sc-88767-SH and PLEKHG5 shRNA (h) Lentiviral Particles: sc-88767-V as alternate gene silencing products.

For independent verification of PLEKHG5 (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-88767A and sc-88767B.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

PLEKHG5 siRNA (h) is recommended for the inhibition of PLEKHG5 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

PLEKHG5 (5A9): sc-517108 is recommended as a control antibody for monitoring of PLEKHG5 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 PLEKHG5 gene expression knockdown using RT-PCR Primer: PLEKHG5 (h)-PR: sc-88767-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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