SANTA CRUZ BIOTECHNOLOGY, INC.

Arhgef5 siRNA (m): sc-141228

BACKGROUND

Rho GTPases, which play fundamental roles in numerous cellular processes, are initiated by external stimuli that signal though G-protein coupled receptors. ARHGEF5 (Rho guanine nucleotide exchange factor (GEF) 5), also known as P60 or transforming immortalized mammary oncogene (TIM), is a 1597 amino acid cytoplasmic protein. ARHGEF5 is believed to form a complex with G proteins and be involved in the initiation of Rho-dependent signals. It has also been suggested that ARHGEF5 may be involved in the organization of the cytoskeleton. ARHGEF5 contains one PH domain, one DH domain and one SH3 domain. Existing as two isoforms produced by alternative splicing, ARHGEF5 is mainly expressed in pancreas, liver, kidney, placenta and lung.

REFERENCES

1. Chan, A.M., et al. 1994. Expression cDNA cloning of a novel oncogene with sequence similarity to regulators of small GTP-binding proteins. Oncogene 9: 1057-1063.
2. Takai, S., et al. 1995. Assignment of the human TIM proto-oncogene to 7q33→q35. Cancer Genet. Cytogenet. 83: 87-89.
3. Snyder, J.T., et al. 2002. Structural basis for the selective activation of Rho GTPases by Dbl exchange factors. Nat. Struct. Biol. 9: 468-475.
4. Umetsu, D.T., et al. 2004. Regulation of tolerance in the respiratory tract: TIM-1, hygiene, and the environment. Ann. N.Y. Acad. Sci. 1029: 88-93.
5. Zhang, Y., et al. 2005. Time-resolved mass spectrometry of tyrosine phosphorylation sites in the epidermal growth factor receptor signaling network reveals dynamic modules. Mol. Cell. Proteomics 4: 1240-1250.
6. Online Mendelian Inheritance in Man, OMIM™. 2006. Johns Hopkins University, Baltimore, MD. MIM Number: 600888. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: Arhgef5 (mouse) mapping to 6 B2.1.

PRODUCT

Arhgef5 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 Arhgef5 shRNA Plasmid (m): sc-141228-SH and Arhgef5 shRNA (m) Lentiviral Particles: sc-141228-V as alternate gene silencing products.

For independent verification of Arhgef5 (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-141228A, sc-141228B and sc-141228C.

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

Arhgef5 siRNA (m) is recommended for the inhibition of Arhgef5 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

ARHGEF5 (4-YD14): sc-134269 is recommended as a control antibody for monitoring of Arhgef5 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 Arhgef5 gene expression knockdown using RT-PCR Primer: Arhgef5 (m)-PR: sc-141228-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.