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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

SANTA CRUZ BIOTECHNOLOGY, INC.

NET1 siRNA (h): sc-41726

BACKGROUND

Numerous cellular functions such as proliferation, differentiation, apoptosis, vesicular trafficking, nuclear transport and cytoskeletal organization are controlled by GTPases. It has become increasingly clear that GTPases act in cascades in which their activities are linked by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). Researchers looking for new epithelial cell-specific oncogenes using a highly efficient cDNA expression cloning system have isolated the Ost oncogene from rat osteosarcoma cells. The Ost proto-oncogene protein contains DH and PH domains, catalyzes guanine nucleotide exchange on RhoA and Cdc42 and interacts specifically with the GTP-bound form of Rac1. The related NET1 protein also contains a DH domain and is ubiquitously expressed in a variety of tissues. Overexpression of NET1 in NIH/3T3 cells results in altered growth properties and tumorigenesis when injected into nude mice.

REFERENCES

1. Miki, T., et al. 1991. Development of a highly efficient expression cDNA cloning system: application to oncogene isolation. Proc. Natl. Acad. Sci. USA 88: 5167-5171.
2. Ron, D., et al. 1991. A region of proto-dbl essential for its transforming activity shows sequence similarity to a yeast cell cycle gene, CDC24, and the human breakpoint cluster gene, bcr. New Biol. 3: 372-379.
3. Mayer, B.J., et al. 1993. A putative modular domain present in diverse signaling proteins. Cell 73: 629-630.
4. Boguski, M.S., et al. 1993. Proteins regulating Ras and its relatives. Nature 366: 643-654.
5. Horii, Y., et al. 1994. A novel oncogene, ost, encodes a guanine nucleotide exchange factor that potentially links Rho and Rac signaling pathways. EMBO J. 13: 4776-4786.
6. Hart, M.J., et al. 1994. Cellular transformation and guanine nucleotide exchange activity are catalyzed by a common domain on the dbl oncogene product. J. Biol. Chem. 269: 62-65.
7. Chant, J., et al. 1995. GTPase cascades choreographing cellular behavior: movement, morphogenesis, and more. Cell 81: 1-4.

CHROMOSOMAL LOCATION

Genetic locus: NET1 (human) mapping to 10p15.1.

PRODUCT

NET1 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 NET1 shRNA Plasmid (h): sc-41726-SH and NET1 shRNA (h) Lentiviral Particles: sc-41726-V as alternate gene silencing products.

For independent verification of NET1 (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-41726A, sc-41726B and sc-41726C.

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

NET1 siRNA (h) is recommended for the inhibition of NET1 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

NET1 (G-4): sc-271941 is recommended as a control antibody for monitoring of NET1 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 NET1 gene expression knockdown using RT-PCR Primer: NET1 (h)-PR: sc-41726-PR (20 µl). 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.