SANTA CRUZ BIOTECHNOLOGY, INC.

ANKRD28 siRNA (h): sc-78515

BACKGROUND

Ankyrins are membrane adaptor molecules that play important roles in coupling integral membrane proteins to the spectrin-based cytoskeleton network. Mutations of ankyrin genes lead to severe genetic diseases, such as fatal cardiac arrhythmias and hereditary spherocytosis. ANKRD28 (Ankyrin repeat domain-containing protein 28), also known as PITK, is a 1,086 amino acid protein that localizes to the nucleus and contains 27 ankyrin repeats. Expressed in a variety of tissues, ANKRD28 functions to selectively inhibit the activity of the PP1 holoenzyme (a trimeric complex that exhibits phosphatase activity and regulates a broad range of cellular functions). Specifically, ANKRD28 targets the catalytic subunit of the PP1 complex to the nucleus and promotes the PP1-dependent dephosphorylation of hnRNP K, a transcriptional regulator. ANKRD28 may also function as a fusion partner for Nup98, an event that may lead to hematologic malignancies, suggesting a role for ANKRD28 in tumorigenesis. Two isoforms of ANKRD28 exist due to alternative splicing events.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 611122. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Kwiek, N.C., et al. 2006. PITK, a PP1 targeting subunit that modulates the phosphorylation of the transcriptional regulator hnRNP K. Cell. Signal. 18: 1769-1778.
3. Kwiek, N.C., et al. 2007. Dual kinase-mediated regulation of PITK by CaMKII and GSK3. Cell. Signal. 19: 593-599.
4. Ishikawa, M., et al. 2007. A novel gene, ANKRD28 on 3p25, is fused with NUP98 on 11p15 in a cryptic 3-way translocation of t(3;5;11)(p25;q35;p15) in an adult patient with myelodysplastic syndrome/acute myelogenous leukemia. Int. J. Hematol. 86: 238-245.
5. Stefansson, B., et al. 2008. Protein phosphatase 6 regulatory subunits composed of ankyrin repeat domains. Biochemistry 47: 1442-1451.
6. Kiyokawa, E. and Matsuda, M. 2009. Regulation of focal adhesion and cell migration by ANKRD28-DOCK180 interaction. Cell Adh. Migr. 3: 281-284.

CHROMOSOMAL LOCATION

Genetic locus: ANKRD28 (human) mapping to 3p25.1.

PRODUCT

ANKRD28 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 ANKRD28 shRNA Plasmid (h): sc-78515-SH and ANKRD28 shRNA (h) Lentiviral Particles: sc-78515-V as alternate gene silencing products.

For independent verification of ANKRD28 (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-78515A, sc-78515B and sc-78515C.

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

ANKRD28 siRNA (h) is recommended for the inhibition of ANKRD28 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

ANKRD28/44/52 (G-6): sc-393032 is recommended as a control antibody for monitoring of ANKRD28 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 ANKRD28 gene expression knockdown using RT-PCR Primer: ANKRD28 (h)-PR: sc-78515-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.