SANTA CRUZ BIOTECHNOLOGY, INC.

VAC14 siRNA (h): sc-72206

BACKGROUND

Phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2) is a signaling molecule that exists as a minor component of cell membranes and is essential for the distinguishing of cellular compartments. The synthesis of PI(3,5)P2 is regulated by a number of proteins that are involved in intracellular trafficking and assembly events throughout the cell. VAC14, also known as TAX1BP2 (Tax1-binding protein 2) or TRX, is a 782 amino acid protein that contains 6 HEAT repeats and exists as part of a regulatory complex with FIG4. Expressed ubiquitously, VAC14 works with FIG4 to control the synthesis of PI(3,5)P2, specifically mediating the activation of PIP5KIII, a kinase involved in the regulation of PI(3,5)P2 activity. The gene encoding VAC14 maps to human chromosome 16, which houses over 900 genes and comprises nearly 3% of the human genome.

REFERENCES

1. Mireskandari, A., et al. 1996. Isolation of a cDNA clone, TRX encoding a human T-cell lymphotrophic virus type-I Tax1 binding protein. Biochim. Biophys. Acta 1306: 9-13.
2. Sbrissa, D., et al. 2004. A mammalian ortholog of *Saccharomyces cerevisiae* Vac14 that associates with and up-regulates PIKfyve phosphoinositide 5-kinase activity. Mol. Cell. Biol. 24: 10437-10447.
3. Lemaire, J.F. and McPherson, P.S. 2006. Binding of Vac14 to neuronal nitric oxide synthase: characterisation of a new internal PDZ-recognition motif. FEBS Lett. 580: 6948-6954.
4. Ching, Y.P., et al. 2006. The retroviral oncoprotein Tax targets the coiled-coil centrosomal protein TAX1BP2 to induce centrosome overduplication. Nat. Cell Biol. 8: 717-724.
5. Sbrissa, D., et al. 2007. Core protein machinery for mammalian phosphatidylinositol 3,5-bisphosphate synthesis and turnover that regulates the progression of endosomal transport. Novel Sac phosphatase joins the ArPIKfyve-PIKfyve complex. J. Biol. Chem. 282: 23878-23891.
6. Zhang, Y., et al. 2007. Loss of Vac14, a regulator of the signaling lipid phosphatidylinositol 3,5-bisphosphate, results in neurodegeneration in mice. Proc. Natl. Acad. Sci. USA 104: 17518-17523.

CHROMOSOMAL LOCATION

Genetic locus: VAC14 (human) mapping to 16q22.1.

PRODUCT

VAC14 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 VAC14 shRNA Plasmid (h): sc-72206-SH and VAC14 shRNA (h) Lentiviral Particles: sc-72206-V as alternate gene silencing products.

For independent verification of VAC14 (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-72206A, sc-72206B and sc-72206C.

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

VAC14 siRNA (h) is recommended for the inhibition of VAC14 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

VAC14 (C-10): sc-271831 is recommended as a control antibody for monitoring of VAC14 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 VAC14 gene expression knockdown using RT-PCR Primer: VAC14 (h)-PR: sc-72206-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.