SANTA CRUZ BIOTECHNOLOGY, INC.

SPAK siRNA (m): sc-76548

BACKGROUND

The phosphorylation and dephosphorylation of proteins on serine and threonine residues is an essential means of regulating a broad range of cellular functions in eukaryotes, including cell division, homeostasis and apoptosis. A group of proteins that are intimately involved in this process are the serine/threonine (Ser/Thr) protein kinases. SPAK, also known as STK39 (serine threonine kinase 39), DCHT or PASK, is a 547 amino acid protein that localizes to both the cytoplasm and the nucleus and contains one protein kinase domain. Expressed predominately in pancreas, brain, heart, lung, liver and testis, SPAK functions as a Ser/Thr protein kinase that catalyzes the ATP-dependent phosphorylation of target proteins and is thought to be involved in mediating stress-activated signals. The gene encoding SPAK maps to human chromosome 2q24.3, which houses over 1,400 genes and comprises nearly 8% of the human genome.

REFERENCES

1. Johnston, A.M., et al. 2000. SPAK, a STE20/SPS1-related kinase that activates the p38 pathway. Oncogene 19: 4290-4297.
2. Qi, H., et al. 2001. Androgens induce expression of SPAK, a STE20/SPS1-related kinase, in LNCaP human prostate cancer cells. Mol. Cell. Endocrinol. 182: 181-192.
3. Dowd, B.F., et al. 2003. PASK (proline-alanine-rich STE20-related kinase), a regulatory kinase of the Na-K-Cl cotransporter (NKCC1). J. Biol. Chem. 278: 27347-27353.
4. Piechotta, K., et al. 2003. Characterization of the interaction of the stress kinase SPAK with the Na^+-K^+-$2Cl^-$ cotransporter in the nervous system: evidence for a scaffolding role of the kinase. J. Biol. Chem. 278: 52848-52856.
5. Online Mendelian Inheritance in Man, OMIM™. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 607648. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
6. Moriguchi, T., et al. 2005. WNK1 regulates phosphorylation of cation-chloride-coupled cotransporters via the STE20-related kinases, SPAK and OSR1. J. Biol. Chem. 280: 42685-42693.

CHROMOSOMAL LOCATION

Genetic locus: Stk39 (mouse) mapping to 2 C1.3.

PRODUCT

SPAK 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 SPAK shRNA Plasmid (m): sc-76548-SH and SPAK shRNA (m) Lentiviral Particles: sc-76548-V as alternate gene silencing products.

For independent verification of SPAK (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-76548A, sc-76548B and sc-76548C.

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

SPAK siRNA (m) is recommended for the inhibition of SPAK 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SPAK gene expression knockdown using RT-PCR Primer: SPAK (m)-PR: sc-76548-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Feng, X., et al. 2015. Aldosterone modulates thiazide-sensitive sodium chloride cotransporter abundance via DUSP6-mediated ERK1/2 signaling pathway. Am. J. Physiol. Renal Physiol. 308: F1119-F1127.

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.