SANTA CRUZ BIOTECHNOLOGY, INC.

ChoK siRNA (h): sc-38965

BACKGROUND

The major pathway for the biosynthesis of phosphatidylcholine occurs via the CDP-choline pathway. Choline kinase, the initial enzyme in the sequence, plays a role in cell growth proliferation. Hemicholinium-3 (HC-3), an inhibitor for Choline kinase (also known as ChoK and CKI), drastically reduces entry into S phase after stimulation by growth factors. In Ras-transformed cells, an increased level of phosphorylcholine (PCho) results from the consecutive activation of phospholipase D (PLD) and ChoK. ChoK and its product, PCho, have been implicated in human carcinogenesis, including the development of human breast cancer, and ChoK dysregulation is found in a variety of human tumors such as lung, colorectal and prostate tumors. The human Choline kinase gene maps to chromosome 11q13.2.

REFERENCES

1. Jimenez, B., et al. 1995. Generation of phosphorylcholine as an essential event in the activation of Raf-1 and MAP-kinases in growth factors-induced mitogenic stimulation. J. Cell. Biochem. 57: 141-149.
2. Hernandez-Alcoceba, R., et al. 1997. Choline kinase inhibitors as a novel approach for antiproliferative drug design. Oncogene 15: 2289-2301.
3. Hernandez-Alcoceba, R., et al. 1999. *In vivo* anti-tumor activity of choline kinase inhibitors: a novel target for anticancer drug discovery. Cancer Res. 59: 3112-3118.
4. Lucas, L., et al. 2001. Modulation of phospholipase D by hexadecylphosphorylcholine: a putative novel mechanism for its antitumoral activity. Oncogene 20: 1110-1117.
5. Ramirez de Molina, A., et al. 2002. Increased choline kinase activity in human breast carcinomas: clinical evidence for a potential novel antitumor strategy. Oncogene 21: 4317-4322.
6. Ramirez de Molina, A., et al. 2002. Overexpression of choline kinase is a frequent feature in human tumor-derived cell lines and in lung, prostate, and colorectal human cancers. Biochem. Biophys. Res. Commun. 296: 580-583.
7. LocusLink Report (LocusID: 1119). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: CHKA (human) mapping to 11q13.2.

PRODUCT

ChoK 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 ChoK shRNA Plasmid (h): sc-38965-SH and ChoK shRNA (h) Lentiviral Particles: sc-38965-V as alternate gene silencing products.

For independent verification of ChoK (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-38965A, sc-38965B and sc-38965C.

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

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

ChoK (B-8): sc-376489 is recommended as a control antibody for monitoring of ChoK 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 ChoK gene expression knockdown using RT-PCR Primer: ChoK (h)-PR: sc-38965-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.