SANTA CRUZ BIOTECHNOLOGY, INC.

CHPT1 siRNA (h): sc-95799

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The Power to Question

BACKGROUND

CHPT1 (cholinephosphotransferase 1), also known as AAPT1-like protein and Diacylglycerol cholinephosphotransferase 1, is a 406 amino acid multi-pass membrane protein that is localized to the golgi apparatus. By catalyzing the phosphatidylcholine biosynthesis from CDP-choline, it plays an essential role in the formation and maintenance of vesicular membranes. CHPT1 is most abundant in testis, as well as small intestine, heart, colon, spleen and prostate. Expression of CHPT1 is increased in cancerous breast cells as compared to normal breast cell lines and it has been determined that the CHPT1 gene exhibits mutations within the cancerous cells. Interestingly, exposure to mustard gas significantly decreases CHPT1 gene expression and activity, an event that may play an important role in the development of acute respiratory distress syndrome (ARDS). There are two isoforms of CHPT1 that are produced as a result of alternative splicing events.

REFERENCES

1. Toback, F.G. 1984. Phosphatidylcholine metabolism during renal growth and regeneration. Am. J. Physiol. 246: F249-F259.
2. McMaster, C.R., et al. 1997. CDP-choline:1,2-diacylglycerol cholinephosphotransferase. Biochim. Biophys. Acta 1348: 100-110.
3. Henneberry, A.L., et al. 1999. Cloning and expression of a human choline/ethanolaminephosphotransferase: synthesis of phosphatidylcholine and phosphatidylethanolamine. Biochem. J. 339: 291-298.
4. Henneberry, A.L., et al. 2000. Cloning, genomic organization, and characterization of a human cholinephosphotransferase. J. Biol. Chem. 275: 29808-29815.
5. Ghosh, A., et al. 2002. Differential expression of cholinephosphotransferase in normal and cancerous human mammary epithelial cells. Biochem. Biophys. Res. Commun. 297: 1043-1048.
6. Cui, Z., et al. 2002. Phosphatidylcholine and cell death. Biochim. Biophys. Acta 1585: 87-96.
7. Vaandrager, A.B., et al. 2002. Effect of ceramides on phospholipid biosynthesis and its implication for apoptosis. Subcell. Biochem. 36: 207-227.

CHROMOSOMAL LOCATION

Genetic locus: CHPT1 (human) mapping to 12q23.2.

PRODUCT

CHPT1 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 CHPT1 shRNA Plasmid (h): sc-95799-SH and CHPT1 shRNA (h) Lentiviral Particles: sc-95799-V as alternate gene silencing products.

For independent verification of CHPT1 (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-95799A, sc-95799B and sc-95799C.

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

CHPT1 siRNA (h) is recommended for the inhibition of CHPT1 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

CHPT1 (F-7): sc-515577 is recommended as a control antibody for monitoring of CHPT1 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 CHPT1 gene expression knockdown using RT-PCR Primer: CHPT1 (h)-PR: sc-95799-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.