SANTA CRUZ BIOTECHNOLOGY, INC.

CYP2A6 siRNA (h): sc-105253

BACKGROUND

The cytochrome P450 proteins (CYPs) are monooxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. P450 enzymes are classified into subfamilies based on their sequence similarities. CYP2A6 is a liver enzyme that metabolizes a number of drugs and a variety of procarcinogens, though it is primarily responsible for the metabolism of nicotine, the major addictive agent in tobacco. CYP2A6 inactivates nicotine to cotinine, and then cotinine to 3-hydroxycotinine. Differences in CYP2A6 genotypes are related to nicotine dependence, and may influence smoking habits and withdrawal symptoms in individuals that are quitting smoking. This suggests that an individualized smoking cessation program may be designed based on CYP2A6 genotypes.

REFERENCES

1. Nakajima, M., et al. 2004. Novel human CYP2A6 alleles confound gene deletion analysis. FEBS Lett. 569: 75-81.
2. Kimura, M., et al. 2005. CYP2A6 is a principal enzyme involved in hydroxylation of 1,7-dimethylxanthine, a main caffeine metabolite, in humans. Drug Metab. Dispos. 33: 1361-1366.
3. Kumarakulasingham, M., et al. 2005. Cytochrome P450 profile of colorectal cancer: identification of markers of prognosis. Clin. Cancer Res. 11: 3758-3765.
4. Swan, G.E., et al. 2005. Nicotine metabolism: the impact of CYP2A6 on estimates of additive genetic influence. Pharmacogenet. Genomics 15: 115-125.
5. von Weymarn, L.B., et al. 2005. Inactivation of CYP2A6 and CYP2A13 during nicotine metabolism. J. Pharmacol. Exp. Ther. 316: 295-303.
6. Benowitz, N.L., et al. 2006. CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. Clin. Pharmacol. Ther. 80: 457-467.
7. Ozaki, S., et al. 2006. Smoking cessation program and CYP2A6 polymorphism. Front. Biosci. 11: 2590-2597.

CHROMOSOMAL LOCATION

Genetic locus: CYP2A6 (human) mapping to 19q13.2.

PRODUCT

CYP2A6 siRNA (h) is a pool of 2 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 CYP2A6 shRNA Plasmid (h): sc-105253-SH and CYP2A6 shRNA (h) Lentiviral Particles: sc-105253-V as alternate gene silencing products.

For independent verification of CYP2A6 (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-105253A and sc-105253B.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

CYP2A6 siRNA (h) is recommended for the inhibition of CYP2A6 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

CYP2A6 (F16 P2 D8): sc-53615 is recommended as a control antibody for monitoring of CYP2A6 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 CYP2A6 gene expression knockdown using RT-PCR Primer: CYP2A6 (h)-PR: sc-105253-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.