SANTA CRUZ BIOTECHNOLOGY, INC.

CYP46 siRNA (m): sc-77076

BACKGROUND

Cytochrome P450 proteins are heme-thiolate monooxygenases that mediate NADPH-dependent electron transport and function to oxidize a variety of structurally unrelated compounds, including steroids, fatty acids and xenobiotics. Specifically, Cytochrome P450s are responsible for metabolizing arachidonic acid to hydroxyeicosatetraenoic acid (a regulator of blood pressure) and epoxyeicosatrienoic acid (a molecule involved in signaling events). CYP46, also known as CYP46A1 (cytochrome P450, family 46, subfamily A, polypeptide 1) or CP46, is a 500 amino acid protein that localizes to the endoplasmic reticulum and shares 95% sequence identity with its mouse counterpart. Expressed predominately in brain tissue, CYP46 catalyzes the conversion of cholesterol into 24S-hydroxycholesterol and, to a lesser extent, 25-hydroxycholesterol, thereby playing an important role in cholesterol homeostasis and turnover. Variations in the gene encoding CYP46 that influence brain cholesterol metabolism are associated with an increased risk for Alzheimer's disease (AD).

REFERENCES

1. Björkhem, I., et al. 1998. Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. J. Lipid Res. 39: 1594-1600.
2. Online Mendelian Inheritance in Man, OMIM™. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 604087. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Papassotiropoulos, A., et al. 2005. A cluster of cholesterol-related genes confers susceptibility for Alzheimer's disease. J. Clin. Psychiatry 66: 940-947.
4. Golanska, E., et al. 2005. CYP46: a risk factor for Alzheimer's disease or a coincidence? Neurosci. Lett. 383: 105-108.
5. Wang, F. and Jia, J. 2007. Polymorphisms of cholesterol metabolism genes CYP46 and ABCA1 and the risk of sporadic Alzheimer's disease in Chinese. Brain Res. 1147: 34-38.

CHROMOSOMAL LOCATION

Genetic locus: Cyp46a1 (mouse) mapping to 12 F1.

PRODUCT

CYP46 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 CYP46 shRNA Plasmid (m): sc-77076-SH and CYP46 shRNA (m) Lentiviral Particles: sc-77076-V as alternate gene silencing products.

For independent verification of CYP46 (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-77076A, sc-77076B and sc-77076C.

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

CYP46 siRNA (m) is recommended for the inhibition of CYP46 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.

GENE EXPRESSION MONITORING

CYP46 (1F11): sc-136148 is recommended as a control antibody for monitoring of CYP46 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.

T-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CYP46 gene expression knockdown using RT-PCR Primer: CYP46 (m)-PR: sc-77076-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.