

OAT3 siRNA (h): sc-96554

BACKGROUND

The organic anion transporter (OAT) family of proteins mediate the secretion of exogenous and endogenous metabolites from tissues throughout the body. OAT1 (organic anion transporter 1), a 563 amino acid protein, and OAT3 (organic anion transporter 3), a 542 amino acid protein, are two members of the OAT family and are highly expressed in kidneys. Localized specifically to the basolateral membrane, OAT1 and OAT3 are involved in the elimination of toxic organic anions, such as benzylpenicillin and cimetidine, from proximal renal tubules. Via their ability to remove anions from renal tissues, OAT1 and OAT3 are able to regulate the amount of toxins within the kidneys. Additionally, OAT1 functions as an organic anion exchanger that couples the uptake of one organic anion molecule with the efflux of one endogenous dicarboxylic acid molecule, such as ketoglutarate. Four isoforms of OAT1 and three isoforms of OAT3 are expressed due to alternative splicing events.

REFERENCES

1. Lu, R., et al. 1999. Cloning of the human kidney PAH transporter: narrow substrate specificity and regulation by protein kinase C. *Am. J. Physiol.* 276: F295-F303.
2. Race, J.E., et al. 1999. Molecular cloning and characterization of two novel human renal organic anion transporters (hOAT1 and hOAT3). *Biochem. Biophys. Res. Commun.* 255: 508-514.
3. Bahn, A., et al. 2000. Genomic structure and *in vivo* expression of the human organic anion transporter 1 (hOAT1) gene. *Biochem. Biophys. Res. Commun.* 275: 623-630.
4. Sun, W., et al. 2001. Isolation of a family of organic anion transporters from human liver and kidney. *Biochem. Biophys. Res. Commun.* 283: 417-422.

CHROMOSOMAL LOCATION

Genetic locus: SLC22A8 (human) mapping to 11q12.3.

PRODUCT

OAT3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see OAT3 shRNA Plasmid (h): sc-96554-SH and OAT3 shRNA (h) Lentiviral Particles: sc-96554-V as alternate gene silencing products.

For independent verification of OAT3 (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-96554A, sc-96554B and sc-96554C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

OAT3 siRNA (h) is recommended for the inhibition of OAT3 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 μ M in 66 μ l. 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

OAT3 (3C11): sc-293264 is recommended as a control antibody for monitoring of OAT3 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 OAT3 gene expression knockdown using RT-PCR Primer: OAT3 (h)-PR: sc-96554-PR (20 μ l, 443 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ng, H.Y., et al. 2014. Indoxyl sulfate downregulates expression of Mas receptor via OAT3/AhR/Stat3 pathway in proximal tubular cells. *PLoS ONE* 9: e91517.
2. Yisireyili, M., et al. 2014. Indoxyl sulfate-induced activation of (pro)renin receptor promotes cell proliferation and tissue factor expression in vascular smooth muscle cells. *PLoS ONE* 9: e109268.

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.