

UGT1A4 siRNA (h): sc-44472

BACKGROUND

Glucuronidation, an important bile acid detoxification pathway, is catalyzed by enzymes belonging to the UDP-glucuronosyltransferase (UGT) superfamily. UGT genes are classified into the UGT1A and UGT2B subfamilies. Although each subfamily and each isoform shows tissue-specific patterns of distribution, the underlying mechanisms for this tissue specificity have not been fully elucidated. The human UDP-glucuronosyltransferase 1 (UGT1) locus encodes at least ten UGT1A proteins (UGT1A1-UGT1A10) that play a prominent role in drug and xenobiotic metabolism. Research indicates that nuclear receptors such as pregnane X receptor (PXR), constitutive androstane receptor (CAR) and peroxisome proliferator-activated receptor (PPAR) can regulate UGTs, which may contribute to the tissue-specific expression pattern of UGTs. Deficiency in the expression and/or activity of UGTs may lead to genetic and acquired diseases such as Crigler-Najjar syndrome and Gilbert syndrome. Based on their ability to catalyze the glucuronidation of xenobiotics and endobiotics, UGTs play a critical role in hormonal homeostasis, energy metabolism, bilirubin clearance and xenobiotic detoxification.

REFERENCES

1. Moghrabi, N., Sutherland, L., Wooster, R., Povey, S., Boxer, M. and Burchell, B. 1992. Chromosomal assignment of human phenol and bilirubin UDP-glucuronosyltransferase genes (UGT1A-subfamily). *Ann. Hum. Genet.* 56: 81-91.
2. Owens, I.S., Ritter, J.K., Yeatman, M.T. and Chen, F. 1996. The novel UGT1 gene complex links bilirubin, xenobiotics and therapeutic drug metabolism by encoding UDP-glucuronosyltransferase isozymes with a common carboxyl terminus. *J. Pharmacokinet. Biopharm.* 24: 491-508.
3. Ciotti, M., Obaray, R., Martin, M.G. and Owens, I.S. 1997. Genetic defects at the UGT1 locus associated with Crigler-Najjar type I disease, including a prenatal diagnosis. *Am. J. Med. Genet.* 68: 173-178.
4. Strassburg, C.P., Manns, M.P. and Tukey, R.H. 1997. Differential downregulation of the UDP-glucuronosyltransferase 1A locus is an early event in human liver and biliary cancer. *Cancer Res.* 57: 2979-2985.
5. Thomas, S.S., Li, S.S., Lampe, J.W., Potter, J.D. and Bigler, J. 2006. Genetic variability, haplotypes and htSNPs for exons 1 at the human UGT1A locus. *Hum. Mutat.* 27: 717.

CHROMOSOMAL LOCATION

Genetic locus: UGT1A4 (human) mapping to 2q37.1.

PRODUCT

UGT1A4 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UGT1A4 shRNA Plasmid (h): sc-44472-SH and UGT1A4 shRNA (h) Lentiviral Particles: sc-44472-V as alternate gene silencing products.

For independent verification of UGT1A4 (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-44472A and sc-44472B.

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

UGT1A4 siRNA (h) is recommended for the inhibition of UGT1A4 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

UGT1A (B-4): sc-271268 is recommended as a control antibody for monitoring of UGT1A4 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 UGT1A4 gene expression knockdown using RT-PCR Primer: UGT1A4 (h)-PR: sc-44472-PR (20 μ l). 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.