SANTA CRUZ BIOTECHNOLOGY, INC.

ATP7B siRNA (m): sc-44492

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

The copper efflux transporters ATP7A and ATP7B sequester intracellular copper into the vesicular secretory pathway for export from the cell. ATP7A functions as a transmembrane copper-translocating P-type ATPase and plays a vital role in systemic copper absorption in the gut and copper reabsorption in the kidney. Polarized epithelial cells such as Madin-Darby canine kidney cells are a physiologically relevant model for systemic copper absorption and reabsorption *in vivo*. Although ATP7A is not detectable in most normal tissues, it is expressed in a considerable fraction of many common tumor types. Increased expression of ATP7A renders cells resistant to cisplatin and carboplatin. Mutations in the ATP7A gene result in Menkes disease, which is fatal in early childhood. Mutations in the ATP7B gene lead to the autosomal recessive disorder Wilson disease, characterized by neurological symptoms and hepatic damage.

REFERENCES

1. Samimi, G., et al. 2003. Increase in expression of the copper transporter ATP7A during platinum drug-based treatment is associated with poor survival in ovarian cancer patients. Clin. Cancer Res. 9: 5853-5859.
2. Samimi, G., et al. 2004. Modulation of the cellular pharmacology of cisplatin and its analogs by the copper exporters ATP7A and ATP7B. Mol. Pharmacol. 66: 25-32.
3. Greenough, M., et al. 2004. Signals regulating trafficking of Menkes (MNK; ATP7A) copper-translocating P-type ATPase in polarized MDCK cells. Am. J. Physiol., Cell Physiol. 287: C1463-C1471.
4. Song, I.S., et al. 2004. Role of human copper transporter Ctr1 in the transport of platinum-based antitumor agents in cisplatin-sensitive and cisplatin-resistant cells. Mol. Cancer Ther. 3: 1543-1549.
5. van Dongen, E.M., et al. 2004. Copper-dependent protein-protein interactions studied by yeast two-hybrid analysis. Biochem. Biophys. Res. Commun. 323: 789-795.
6. Huster, D., et al. 2004. Rapid detection of mutations in Wilson disease gene ATP7B by DNA strip technology. Clin. Chem. Lab. Med. 42: 507-510.

CHROMOSOMAL LOCATION

Genetic locus: Atp7b (mouse) mapping to 8 A2.

PRODUCT

ATP7B 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 ATP7B shRNA Plasmid (m): sc-44492-SH and ATP7B shRNA (m) Lentiviral Particles: sc-44492-V as alternate gene silencing products.

For independent verification of ATP7B (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-44492A, sc-44492B and sc-44492C.

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

ATP7B siRNA (m) is recommended for the inhibition of ATP7B 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

ATP7B (A-11): sc-373964 is recommended as a control antibody for monitoring of ATP7B 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 ATP7B gene expression knockdown using RT-PCR Primer: ATP7B (m)-PR: sc-44492-PR (20 µl, 419 bp). 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.