

MRP3 siRNA (m): sc-40749

BACKGROUND

The two members of the large family of ABC transporters known to confer multidrug resistance in human cancer cells are the Mdr1 P-glycoprotein and the multidrug-resistance protein MRP1. MRP1 is an integral membrane protein that contains an Mdr-like core, an N-terminal membrane-bound region and a cytoplasmic linker, and it is expressed in various cerebral cells, as well as in lung, testis and peripheral blood. The MRP gene family also includes MRP2, which is alternatively designated cMOAT (for canalicular multispecific organic anion transporter) and MRP3, which are both conjugate export pumps expressed predominantly in hepatocytes. MRP2 localizes exclusively to the apical membrane and is constitutively expressed at a high level in normal liver cells. Conversely, MRP3 localizes to the basolateral membrane where it also mediates the transport of the organic anion S-(2,4-dinitrophenyl)-glutathione toward the basolateral side of the membrane. MRP3 is normally expressed at comparatively lower levels than MRP2 and increases only when secretion across the apical membrane by MRP2 is impaired. MRP6 protein is highly expressed in liver and kidney, whereas MRP4 and MRP5 are detected in various tissues yet at much lower levels of expression.

REFERENCES

1. Versantvoort, C.H., et al. 1995. Regulation by glutathione of drug transport in multidrug-resistant human lung tumour cell lines overexpressing multidrug resistance-associated protein. *Br. J. Cancer* 72: 82-89.
2. Keppler, D. and König, J. 1997. Hepatic canalicular membrane 5: expression and localization of the conjugate export pump encoded by the MRP2 (cMRP/cMOAT) gene in liver. *FASEB J.* 11: 509-516.
3. Kool, M., et al. 1997. Analysis of expression of cMOAT (MRP2), MRP3, MRP4 and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. *Cancer Res.* 57: 3537-3547.
4. Bakos, E., et al. 1998. Functional multidrug resistance protein (MRP1) lacking the N-terminal transmembrane domain. *J. Biol. Chem.* 273: 32167-32175.
5. Ortiz, D.F., et al. 1999. MRP3, a new ATP-binding cassette protein localized to the canalicular domain of the hepatocyte. *Am. J. Physiol.* 276: G1493-G1500.

CHROMOSOMAL LOCATION

Genetic locus: MRP3 (mouse) mapping to 11 D.

PRODUCT

MRP3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MRP3 shRNA Plasmid (m): sc-40749-SH and MRP3 shRNA (m) Lentiviral Particles: sc-40749-V as alternate gene silencing products.

For independent verification of MRP3 (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-40749A, sc-40749B and sc-40749C.

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

MRP3 siRNA (m) is recommended for the inhibition of MRP3 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 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

MRP3 (B-2): sc-518159 is recommended as a control antibody for monitoring of MRP3 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 MRP3 gene expression knockdown using RT-PCR Primer: MRP3 (m)-PR: sc-40749-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.