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MLN64 siRNA (h): sc-44439

BACKGROUND

Sterol transport is mediated by vesicles or by soluble protein carriers, such as steroidogenic acute regulatory protein (StAR). StAR is homologous to a family of proteins containing a 200-210 amino acid StAR-related lipid transfer (StART) domain, including StARD3 (also known as MLN64). Amplification of the gene which encodes MLN64 results in overexpression and coamplification with ErbB-2 in breast cancer cell lines. Immunoblot analysis shows expression in most breast cancer cell lines and tissues, as well as in an ovary carcinoma cell line. Immunofluorescence microscopy and mutation analysis shows cytoplasmic expression in condensation sites and perinuclear condensation in breast cancer biopsies. It is suggested that MLN64 acts on late endosome cholesterol traffic, possibly lowering cholesterol by shuttling it to a cytoplasmic receptor site.

REFERENCES

1. Tomasetto, C., et al. 1995. Identification of four novel human genes amplified and overexpressed in breast carcinoma and localized to the q11-q21.3 region of chromosome 17. *Genomics* 28: 367-376.
2. Moog-Lutz, C., et al. 1997. MLN64 exhibits homology with the steroidogenic acute regulatory protein (StAR) and is over-expressed in human breast carcinomas. *Int. J. Cancer* 71: 183-191.
3. Watari, H., et al. 1997. MLN64 contains a domain with homology to the steroidogenic acute regulatory protein (StAR) that stimulates steroidogenesis. *Proc. Natl. Acad. Sci. USA* 94: 8462-8467.
4. Alpy, F., et al. 2001. The steroidogenic acute regulatory protein homolog MLN64, a late endosomal cholesterol-binding protein. *J. Biol. Chem.* 276: 4261-4269.
5. Soccio, R.E., et al. 2002. The cholesterol-regulated StARD4 gene encodes a StAR-related lipid transfer protein with two closely related homologues, StARD5 and StARD6. *Proc. Natl. Acad. Sci. USA* 99: 6943-6948.

CHROMOSOMAL LOCATION

Genetic locus: STARD3 (human) mapping to 17q12.

PRODUCT

MLN64 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 MLN64 shRNA Plasmid (h): sc-44439-SH and MLN64 shRNA (h) Lentiviral Particles: sc-44439-V as alternate gene silencing products.

For independent verification of MLN64 (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-44439A, sc-44439B and sc-44439C.

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 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

MLN64 siRNA (h) is recommended for the inhibition of MLN64 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

MLN64 (H-1): sc-166215 is recommended as a control antibody for monitoring of MLN64 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 MLN64 gene expression knockdown using RT-PCR Primer: MLN64 (h)-PR: sc-44439-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.