

MFG-E8 siRNA (h): sc-43158

BACKGROUND

Human milk-fat globule (MFG) is abundant in human breast milk and is composed of secreted lipids encapsulated by plasma membranes from the epithelial cells of mammary glands. MFG membranes are composed of various glycoproteins that serve as markers for differentiated carcinomas. MFG-E8 (milk fat globule-EGF factor 8), also known as Lactadherin or BA46, is a 387 amino acid peripheral membrane protein that localizes to the membrane of a variety of tissues, including mammary epithelial surfaces, and contains one EGF-like domain and two F5/8 type C domains. Functioning as a specific ligand for Integrin $\beta 5$ and Integrin $\beta 3$, MFG-E8 is thought to be involved in gamete interactions and cell attachment, possibly playing a role in fertilization and apoptosis. Additionally, MFG-E8 binds to rotavirus and inhibits its replication, thereby protecting the cell from viral infection. Overexpression of MFG-E8 is associated with breast cancer, suggesting that MFG-E8 may be related to tumorigenesis.

REFERENCES

1. Newburg, D.S., et al. 1998. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. *Lancet* 351: 1160-1164.
2. Peterson, J.A., et al. 1998. Milk fat globule glycoproteins in human milk and in gastric aspirates of mother's milk-fed preterm infants. *Pediatr. Res.* 44: 499-506.
3. Oshima, K., et al. 2002. Secretion of a peripheral membrane protein, MFG-E8, as a complex with membrane vesicles. *Eur. J. Biochem.* 269: 1209-1218.
4. Hanayama, R., et al. 2002. Identification of a factor that links apoptotic cells to phagocytes. *Nature* 417: 182-187.
5. Ait-Oufella, H., et al. 2007. Lactadherin deficiency leads to apoptotic cell accumulation and accelerated atherosclerosis in mice. *Circulation* 115: 2168-2177.

CHROMOSOMAL LOCATION

Genetic locus: MFG8 (human) mapping to 15q26.1.

PRODUCT

MFG-E8 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 MFG-E8 shRNA Plasmid (h): sc-43158-SH and MFG-E8 shRNA (h) Lentiviral Particles: sc-43158-V as alternate gene silencing products.

For independent verification of MFG-E8 (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-43158A, sc-43158B and sc-43158C.

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

MFG-E8 siRNA (h) is recommended for the inhibition of MFG-E8 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

MFG-E8 (MFG-06): sc-8029 is recommended as a control antibody for monitoring of MFG-E8 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 MFG-E8 gene expression knockdown using RT-PCR Primer: MFG-E8 (h)-PR: sc-43158-PR (20 μ l, 428 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.