

MAGI-3 siRNA (h): sc-42004

BACKGROUND

The membrane-associated guanylate kinase (MAGUK) proteins are concentrated at the membrane-cytoskeletal interface where they facilitate the assembly of multiprotein complexes on the inner surface of the plasma membrane. Three protein-protein interaction modules characteristically define MAGUK-related proteins: the PDZ domain, the SH3 domain and the guanylate kinase (GuK) domain. The closely related MAGUK proteins, MAGI-1, MAGI-2 and MAGI-3, contain the GuK domain and five PDZ domains; however, the SH3 domain is replaced with a WW domain. The transcripts of MAGI-1 are alternatively spliced to produce three distinct proteins having unique carboxy-terminals. Two variants, MAGI-1a and MAGI-1b, are associated with the membrane and cytosolic fractions and are primarily expressed in the brain. The third isoform, MAGI-1c, encodes for a nuclear localization signal that localizes MAGI-1c to the nucleus, and it is primarily expressed in the liver and kidney. MAGI-2 and MAGI-3 are localized to the plasma membrane, and they contribute to protein scaffolding by associating with the protein phosphatase PTEN.

REFERENCES

1. Anderson, J.M. 1996. Cell signalling: MAGUK magic. *Curr. Biol.* 6: 382-384.
2. Dobrosotskaya, I., et al. 1997. MAGI-1, a membrane-associated guanylate kinase with a unique arrangement of protein-protein interaction domains. *J. Biol. Chem.* 272: 31589-31597.
3. Wood, J.D., et al. 1998. Atrophin-1, the DRPLA gene product, interacts with two families of WW domain-containing proteins. *Mol. Cell. Neurosci.* 11: 149-160.
4. Dimitratos, S.D., et al. 1999. Signaling pathways are focused at specialized regions of the plasma membrane by scaffolding proteins of the MAGUK family. *Bioessays* 21: 912-921.
5. Dobrosotskaya, I.Y. and James, G.L. 2000. MAGI-1 interacts with β -catenin and is associated with cell-cell adhesion structures. *Biochem. Biophys. Res. Commun.* 270: 903-909.
6. Wu, Y., et al. 2000. Interaction of the tumor suppressor PTEN/MMAC with a PDZ domain of MAGI-3, a novel membrane-associated guanylate kinase. *J. Biol. Chem.* 275: 21477-21485.

CHROMOSOMAL LOCATION

Genetic locus: MAGI3 (human) mapping to 1p13.2.

PRODUCT

MAGI-3 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 MAGI-3 shRNA Plasmid (h): sc-42004-SH and MAGI-3 shRNA (h) Lentiviral Particles: sc-42004-V as alternate gene silencing products.

For independent verification of MAGI-3 (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-42004A, sc-42004B and sc-42004C.

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

MAGI-3 siRNA (h) is recommended for the inhibition of MAGI-3 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

MAGI-3 (46): sc-136471 is recommended as a control antibody for monitoring of MAGI-3 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 MAGI-3 gene expression knockdown using RT-PCR Primer: MAGI-3 (h)-PR: sc-42004-PR (20 μ l, 551 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.