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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

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.

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.

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.

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.