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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

SANTA CRUZ BIOTECHNOLOGY, INC.

Gem siRNA (m): sc-41720

BACKGROUND

Gem belongs to the Rad/Gem/Kir (RGK) subfamily of Ras-related GTPases, which lack typical C-terminal amino acid motifs for isoprenylation. Rad and Gem bind calmodulin in a Ca^{2+}-dependent manner via this C-terminal extension, involving residues 278–297 in human Rad. High intracellular Gem levels, which interact with intact microtubules and microfilaments, promote profound changes in cell morphology. Ectopic Gem expression is sufficient to stimulate cell flattening and neurite extension in N1E-115 and SH-SY5Y neuroblastoma cells, suggesting a role for Gem in cytoskeletal rearrangement and/or morphological differentiation of neurons. Gem was also observed in developing trigeminal nerve ganglia in 12.5 day mouse embryos, demonstrating that Gem expression is a property of normal ganglionic development. The interaction of Gem with β-subunits regulates Ca^{2+} channel expression at the cell surface. The human Gem gene maps to chromosome 8q22.1.

REFERENCES

1. Bilan, P.J., et al. 1998. The ras-related protein rad associates with the cytoskeleton in a non-lipid-dependent manner. Exp. Cell Res. 242: 391-400.
2. Moyers, J.S., et al. 1998. Effects of phosphorylation on function of the Rad GTPase. Biochem. J. 333: 609-614.
3. Piddini, E., et al. 2001. The Ras-like GTPase Gem is involved in cell shape remodelling and interacts with the novel kinesin-like protein KIF9. EMBO J. 20: 4076-4087.
4. Leone, A., et al. 2001. The Gem GTP-binding protein promotes morphological differentiation in neuroblastoma. Oncogene 20: 3217-3225.
5. Beguin, P., et al. 2001. Regulation of Ca^{2+} channel expression at the cell surface by the small G-protein kir/Gem. Nature 411: 701-706.
6. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 600164. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: Gem (mouse) mapping to 4 A1.

PRODUCT

Gem 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Gem shRNA Plasmid (m): sc-41720-SH and Gem shRNA (m) Lentiviral Particles: sc-41720-V as alternate gene silencing products.

For independent verification of Gem (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-41720A, sc-41720B and sc-41720C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Gem siRNA (m) is recommended for the inhibition of Gem 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 µM in 66 µl. 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

Gem (G-1): sc-166891 is recommended as a control antibody for monitoring of Gem 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Gem gene expression knockdown using RT-PCR Primer: Gem (m)-PR: sc-41720-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension 4temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.