

RGMa siRNA (h): sc-45732

BACKGROUND

The repulsive guidance molecule (RGM) family of proteins are important in the guidance of growth cones of developing neurons. They are repulsive for a group of axons, those from the temporal half of the retina. RGM have been implicated in both axonal guidance and neural tube closure, but unlike ephrins, semaphorins, netrins and slits, no receptor mechanism for RGM activation has been defined. Dorsal root ganglion axons do not respond to RGM but neogenin (a netrin-binding protein which can function as an RGM receptor) expression can spur RGM responsiveness. The RGM proteins are attached to the membrane by a GPI-anchor. Two members of this family, RGMa and RGMb, are expressed in the nervous system. RGMc, also known as hemojuvelin, is a part of the signaling pathway activating hepcidin and works together with hepcidin to restrict iron absorption in the gut. Defects in the gene encoding for RGMc cause the autosomal recessive disorder juvenile hemochromatosis (JH).

REFERENCES

1. Matsunaga, E. and Chedotal, A. 2004. Repulsive guidance molecule/neogenin: a novel ligand-receptor system playing multiple roles in neural development. *Dev. Growth Differ.* 46: 481-486.
2. Matsunaga, E., et al. 2004. RGM and its receptor neogenin regulate neuronal survival. *Nat. Cell Biol.* 6: 749-755.
3. Rajagopalan, S., et al. 2004. Neogenin mediates the action of repulsive guidance molecule. *Nat. Cell Biol.* 6: 756-762.
4. Brinks, H., et al. 2004. The repulsive guidance molecule RGMa is involved in the formation of afferent connections in the dentate gyrus. *J. Neurosci.* 24: 3862-3869.
5. Oldekamp, J., et al. 2004. Expression pattern of the repulsive guidance molecules RGMa, b and c during mouse development. *Gene Expr. Patterns* 4: 283-288.

CHROMOSOMAL LOCATION

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

PRODUCT

RGMa 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 RGMa shRNA Plasmid (h): sc-45732-SH and RGMa shRNA (h) Lentiviral Particles: sc-45732-V as alternate gene silencing products.

For independent verification of RGMa (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-45732A, sc-45732B and sc-45732C.

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

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

RGMa (E-10): sc-393046 is recommended as a control antibody for monitoring of RGMa 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 RGMa gene expression knockdown using RT-PCR Primer: RGMa (h)-PR: sc-45732-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.