SANTA CRUZ BIOTECHNOLOGY, INC.

GPR3 siRNA (m): sc-72174

BACKGROUND

G protein-coupled receptor 3 (GPR3), also designated ACCA orphan receptor, is a 330 amino acid member of the G protein-coupled receptor 1 family. The function of GPR3 is mediated by G proteins which activate adenylate cyclase. GPR3 is a mutli-pass membrane protein that is located on the cellular membrane of cells and is detected at low levels in the eye, kidney, lung, ovary and testis. GPR3 is most highly expressed in the central nervous system, where it stimulates the production of cAMP, leading to neurite outgrowth and myelin inhibition. In oocytes, this control over cAMP production can halt meiosis and prevent progesterone-induced meiotic maturation. Mice deficient for GPR3 are able to reproduce but have no control over the oocyte maturation process, which results in nondeveloping early embryos and fragmented oocytes as the mice age.

REFERENCES

1. Iismaa, T.P., et al. 1995. Isolation and chromosomal localization of a novel human G-protein-coupled receptor (GPR3) expressed predominantly in the central nervous system. Genomics 24: 391-394.
2. Eggerickx, D., et al. 1995. Molecular cloning of an orphan G protein-coupled receptor that constitutively activates adenylate cyclase. Biochem. J. 309: 837-843.
3. Marchese, A., et al. 1995. Cloning of human genes encoding novel G protein-coupled receptors. Genomics 23: 609-618.
4. Song, Z.H., et al. 1996. Molecular cloning and chromosomal localization of human genes encoding three closely related G protein-coupled receptors. Genomics 28: 347-349.
5. Hinckley,M., et al. 2005. The G protein-coupled receptors GPR3 and GPR12 are involved in cAMP signaling and maintenance of meiotic arrest in rodent oocytes. Dev. Biol. 287: 249-261.
6. Feuerstein, P., et al. 2006. Oocyte-cumulus dialog. Gynecol. Obstet. Fertil. 34: 793-800.
7. Tanaka, S., et al. 2007. Neural expression of G protein-coupled receptors GPR3, GPR6, and GPR12 upregulates cyclic AMP levels and promotes neurite outgrowth. J. Biol. Chem. 282: 10506-10515.

CHROMOSOMAL LOCATION

Genetic locus: Gpr3 (mouse) mapping to 4 D2.3.

PRODUCT

GPR3 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 GPR3 shRNA Plasmid (m): sc-72174-SH and GPR3 shRNA (m) Lentiviral Particles: sc-72174-V as alternate gene silencing products.

For independent verification of GPR3 (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-72174A, sc-72174B and sc-72174C.

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

GPR3 siRNA (m) is recommended for the inhibition of GPR3 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

GPR3 (B-5): sc-390276 is recommended as a control antibody for monitoring of GPR3 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 GPR3 gene expression knockdown using RT-PCR Primer: GPR3 (m)-PR: sc-72174-PR (20 µl). 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.