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granuphilin shRNA (h) Lentiviral Particles: sc-45507-V

BACKGROUND

Granuphilin, also designated synaptotagmin-like protein 4 or exophilin 2, is a member of the synaptotagmin-like protein family. It is part of a ternary complex consisting of Syntaxin 1A (STX1A) and Rab 27A. The interaction between granuphilin and Syntaxin 1A on the plasma membrane is regulated by Rab 27A. Granuphilin is a peripheral membrane protein mainly expressed in pancreatic β -cells and in the pituitary gland. It has been detected on secretory granules and close to the plasma membrane. In the pancreas, granuphilin interacts with Insulin-containing vesicles while in both the pancreas and pituitary, granuphilin modulates the secretion of hormones and the exocytosis of dense-core granules. Overexpression of granuphilin enhances basal Insulin secretion but also inhibits high K^+ -induced Insulin secretion. The effect of granuphilin on Insulin secretion may be impaired by a mutation that disrupts the binding to either Rab 27A or Syntaxin 1A, making granuphilin a possible regulator in the exocytotic pathway.

REFERENCES

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3. Izumi, T., Gomi, H. and Torii, S. 2005. Functional analysis of Rab 27A effector granuphilin in Insulin exocytosis. *Methods Enzymol.* 403: 216-229.
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5. Fukuda, M., Imai, A., Nashida, T. and Shimomura, H. 2005. Slp4-a/granuphilin- α interacts with syntaxin-2/3 in a Munc18-2-dependent manner. *J. Biol. Chem.* 280: 39175-39184.

CHROMOSOMAL LOCATION

Genetic locus: SYTL4 (human) mapping to Xq22.1.

PRODUCT

granuphilin shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see granuphilin siRNA (h): sc-45507 and granuphilin shRNA Plasmid (h): sc-45507-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

granuphilin shRNA (h) Lentiviral Particles is recommended for the inhibition of granuphilin expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

granuphilin (C-3): sc-374544 is recommended as a control antibody for monitoring of granuphilin 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor granuphilin gene expression knockdown using RT-PCR Primer: granuphilin (h)-PR: sc-45507-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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