SANTA CRUZ BIOTECHNOLOGY, INC.

GMD siRNA (h): sc-95594

BACKGROUND

GMD (GDP-D-mannose dehydratase), also known as GMDS (GDP-mannose 4,6-dehydratase) or SDR3E1, is a 372 amino acid protein that utilizes NADP as a cofactor to catalyze the conversion of GDP-mannose to GDP-4-keto-6-deoxymannose. GMD mutations are involved in resistance to TRAIL (tumor necrosis factor-related apoptosis-inducing ligand)-induced apoptosis. The gene encoding GMD maps to human chromosome 6, which contains 170 million base pairs and comprises nearly 6% of the human genome. Deletion of a portion of the q arm of chromosome 6 is associated with early onset intestinal cancer, suggesting the presence of a cancer susceptibility locus. Additionally, porphyria cutanea tarda, Parkinson's disease, Stickler syndrome and a susceptibility to bipolar disorder are all associated with genes that map to chromosome 6.

REFERENCES

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2. Sullivan, F.X., et al. 1998. Molecular cloning of human GDP-mannose 4,6-dehydratase and reconstitution of GDP-fucose biosynthesis *in vitro*. J. Biol. Chem. 273: 8193-8202.
3. Ohyama, C., et al. 1998. Molecular cloning and expression of GDP-D-mannose-4,6-dehydratase, a key enzyme for fucose metabolism defective in Lec13 cells. J. Biol. Chem. 273: 14582-14587.
4. Eshel, R., et al. 2001. The FX enzyme is a functional component of lymphocyte activation. Cell. Immunol. 213: 141-148.
5. Bläker, H., et al. 2008. Recurrent deletions at 6q in early age of onset non-HNPCC- and non-FAP-associated intestinal carcinomas. Evidence for a novel cancer susceptibility locus at 6q14-q22. Genes Chromosomes Cancer 47: 159-164.
6. Moriwaki, K., et al. 2009. Deficiency of GMDS leads to escape from NK cell-mediated tumor surveillance through modulation of TRAIL signaling. Gastroenterology 137: 188-198, 198.e1-e2.
7. Aldinger, K.A., et al. 2009. FOXC1 is required for normal cerebellar development and is a major contributor to chromosome 6p25.3 Dandy-Walker malformation. Nat. Genet. 41: 1037-1042.

CHROMOSOMAL LOCATION

Genetic locus: GMDS (human) mapping to 6p25.3.

PRODUCT

GMD 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GMD shRNA Plasmid (h): sc-95594-SH and GMD shRNA (h) Lentiviral Particles: sc-95594-V as alternate gene silencing products.

For independent verification of GMD (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-95594A, sc-95594B and sc-95594C.

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

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

GMD (C-7): sc-515226 is recommended as a control antibody for monitoring of GMD 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 GMD gene expression knockdown using RT-PCR Primer: GMD (h)-PR: sc-95594-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.