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IMPDH shRNA (h) Lentiviral Particles: sc-45679-V

BACKGROUND

A member of the GMPR family, inosine-5'-monophosphate dehydrogenase 1 (IMPDH1) functions in the regulation of cell growth by catalyzing the rate-limiting step in the *de novo* synthesis of guanine nucleotides. IMPDH1 is a ubiquitously expressed homotetramer that plays an important role in cyclic nucleoside metabolism within photoreceptors. Expression of IMPDH1 is the main type found in normal leukocytes, while IMPDH2 predominates in tumors. Mutations in IMPDH1 are associated with the autosomal dominant retinitis pigmentosa type 10 (RP10), as well as the development of malignant tumors. Analysis of mutant IMPDH1 suggests that protein misfolding and aggregation leads to the severe phenotype rather than reduced IMPDH1 activity. Therefore, IMPDH1 may be a potential therapeutic target based upon a strategy combining simultaneous suppression of IMPDH1 transcripts with supplementation of GTP within retinal tissues.

REFERENCES

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2. Bowne, S.J., et al. 2002. Mutations in the inosine monophosphate dehydrogenase 1 gene (IMPDH1) cause the RP10 form of autosomal dominant retinitis pigmentosa. *Hum. Mol. Genet.* 11: 559-568.
3. Pankiewicz, K.W., et al. 2004. Cofactor mimics as selective inhibitors of NAD-dependent inosine monophosphate dehydrogenase (IMPDH)-the major therapeutic target. *Curr. Med. Chem.* 11: 887-900.
4. Wright, D.G., et al. 2004. Effects of the IMP-dehydrogenase inhibitor, Tiazofurin, in bcr-abl positive acute myelogenous leukemia. Part II. *In vitro* studies. *Leuk. Res.* 28: 1137-1143.
5. Aherne, A. et al. 2004. On the molecular pathology of neurodegeneration in IMPDH1-based retinitis pigmentosa. *Hum. Mol. Genet.* 13: 641-650.
6. SWISS-PROT/TrEMBL (P20839). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: IMPDH1 (human) mapping to 7q31.3-q32.

PRODUCT

IMPDH 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 IMPDH siRNA (h): sc-45679 and IMPDH shRNA Plasmid (h): sc-45679-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

IMPDH shRNA (h) Lentiviral Particles is recommended for the inhibition of IMPDH 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

IMPDH (F-6): sc-166551 is recommended as a control antibody for monitoring of IMPDH 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 IMPDH gene expression knockdown using RT-PCR Primer: IMPDH (h)-PR: sc-45679-PR (20 μ l, 540 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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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