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Autotaxin shRNA (h) Lentiviral Particles: sc-44906-V

BACKGROUND

Autotaxin (ATX), also designated ectonucleotide pyrophosphatase/phosphodiesterase 2 (E-NPP 2), is a membrane-bound glycoprotein that cleaves diester bonds for a broad range of substrates. Originally isolated from the human melanoma cell line (A2058), Autotaxin is predominantly expressed in brain, placenta, ovary and small intestine. Autotaxin has significant homology to the cell membrane differentiation antigen PC-1, and is a stimulator of tumor cell motility. It also functions as a catalyst by hydrolytically removing 5'-nucleotides from the 3'-hydroxy termini of 3'-hydroxy-terminated oligonucleotides.

REFERENCES

- Murata, J., et al. 1994. cDNA cloning of the human tumor motility-stimulating protein, autotaxin, reveals a homology with phosphodiesterases. *J. Biol. Chem.* 269: 30479-30484.
- Kawagoe, H., et al. 1995. Molecular cloning and chromosomal assignment of the human brain-type phosphodiesterase I/nucleotide pyrophosphatase gene (PDNP2). *Genomics.* 30: 380-384.
- Lee, HY. et al. 1996. Cloning, chromosomal localization, and tissue expression of autotaxin from human teratocarcinoma cells. *Biochem. Biophys. Res. Commun.* 218: 714-719.
- Mazereeuw-Hautier, J., et al. 2005. Production of lysophosphatidic acid in blister fluid: involvement of a lysophospholipase D activity. *J. Invest. Dermatol.* 125: 421-427.
- Baumforth, K.R., et al. 2005. Induction of autotaxin by the Epstein-Barr virus promotes the growth and survival of Hodgkin lymphoma cells. *Blood.* 106: 2138-2146.
- SWISS-PROT/TrEMBL (Q13822). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: ENPP2 (human) mapping to 8q24.12.

PRODUCT

Autotaxin 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 Autotaxin siRNA (h): sc-44906 and Autotaxin shRNA Plasmid (h): sc-44906-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

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

Autotaxin (E-12): sc-374222 is recommended as a control antibody for monitoring of Autotaxin 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 Autotaxin gene expression knockdown using RT-PCR Primer: Autotaxin (h)-PR: sc-44906-PR (20 μ l, 597 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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