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HPA2 shRNA (h) Lentiviral Particles: sc-43852-V

BACKGROUND

Heparanases degrade heparan sulfate side chains of heparan sulfate proteoglycans (HSPGs) in the extracellular matrix and play an important role in the extravasation of blood-borne tumor cells and inflammatory leukocytes. Upon degradation, heparanases free growth factors and cytokines that stimulate cell proliferation and chemotaxis. Human heparanase 2 (HPA2) and human heparanase 1 (HPA1) are members of the heparanase family. Three alternative splice variants of the HPA2 transcripts encode predicted intracellular membrane-bound proteins of various lengths. HPA2 is expressed in brain, small intestine, prostate, mammary gland, testis, and uterus. While HPA2 is not expressed in normal pancreas, it is expressed in pancreatic tumor cell lines MiaPaca-2 and Panc-1 as well as pancreatic adenocarcinoma. The gene encoding HPA2 maps to human chromosome 10q24.2.

REFERENCES

1. Vlodavsky, I., Fuks, Z., Bar-Ner, M., Ariav, Y. and Schirmmacher, V. 1983. Lymphoma cell mediated degradation of sulfated proteoglycans in the subendothelial extracellular matrix: relationship to tumor cell metastasis. *Cancer Res.* 43: 2704-2711.
2. Bashkin, P., Doctrow, S., Klagsbrun, M., Svahn, C.M., Folkman, J. and Vlodavsky, I., 1989. Basic fibroblast growth factor binds to sunendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. *Biochemistry* 28: 1737-1743.
3. Vlodavsky, I., Korner, G., Ishai-Michaeli, R., Bashkin, P., Bar-Shavit, R. and Fuks, Z., 1990. Extracellular matrix-resident growth factors and enzyme: Possible involvement in tumor metastasis and angiogenesis. *Cancer Metastasis Rev.* 9: 203-226.
4. Vlodavsky, I., Eldor, A., Haimovitz-Friedman, A., Metzner, Y., Ishai-Michaeli, R., Lider, O., Napastek, Y., Cohen, I.R. and Fuks, Z. 1992. Expression of heparanase by platelets and circulating cells of the immune system: possible involvement in diapedesis and extravasation. *Invasion Metastasis* 12: 112-127.

CHROMOSOMAL LOCATION

Genetic locus: HPSE2 (human) mapping to 10q24.2.

PRODUCT

HPA2 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 HPA2 siRNA (h): sc-43852 and HPA2 shRNA Plasmid (h): sc-43852-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

HPA2 shRNA (h) Lentiviral Particles is recommended for the inhibition of heparanase 2 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

HPA2 (C-17): sc-14900 is recommended as a control antibody for monitoring of heparanase 2 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor heparanase 2 gene expression knockdown using RT-PCR Primer: HPA2 (h)-PR: sc-43852-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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.