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HIF PHD3 shRNA (h) Lentiviral Particles: sc-45799-V

BACKGROUND

Prolyl hydroxylase domain proteins PHD1, PHD2 and PHD3 (also designated EGLN2, EGLN1, and EGLN3 respectively) can hydroxylate HIF- α subunits. Hypoxia-inducible factor (HIF) is a transcriptional regulator important in several aspects of oxygen homeostasis. The prolyl hydroxylases catalyze the posttranslational formation of 4-hydroxyproline in HIF- α proteins. PHD1, which is widely expressed but in highest levels in testis, functions as a cellular oxygen sensor and is important in cell growth regulation. PHD1, which can localize to the nucleus or the cytoplasm, is also detected in hormone responsive tissues, such as normal and cancerous mammary, ovarian and prostate epithelium. PHD1 is encoded by EGLN2 which maps to chromosome 19q13.3. PHD2 is regarded as the main cellular oxygen sensor as RNA interference against PHD2, but not PHD1 or PHD3, is enough to stabilize HIF-1 α in normoxia. PHD2, a direct HIF target gene, is expressed mainly in skeletal muscle, heart, kidney and brain. PHD3 may play a role in the regulation of cell growth in muscle cells and in apoptosis in neuronal tissue. PHD3 is widely expressed although the highest levels can be detected in placenta and heart.

REFERENCES

- Appelhoff, R.J., et al. 2004. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J. Biol. Chem.* 279: 38458-38465.
- Aprelikova, O., et al. 2004. Regulation of HIF prolyl hydroxylases by hypoxia-inducible factors. *J. Cell Biochem.* 92: 491-501.
- Marxsen, J.H., et al. 2004. Hypoxia-inducible factor-1 (HIF-1) promotes its degradation by induction of HIF- α -prolyl-4-hydroxylases. *Biochem. J.* 381: 761-767.
- Metzen, E., et al. 2005. Regulation of the prolyl hydroxylase domain protein 2 (Phd2/egln-1) gene: identification of a functional hypoxia-responsive element. *Biochem. J.* 387: 711-717.

CHROMOSOMAL LOCATION

Genetic locus: EGLN3 (human) mapping to 14q13.1.

PRODUCT

HIF PHD3 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 HIF PHD3 siRNA (h): sc-45799 and HIF PHD3 shRNA Plasmid (h): sc-45799-SH as alternate gene silencing products.

APPLICATIONS

HIF PHD3 shRNA (h) Lentiviral Particles is recommended for the inhibition of HIF PHD3 expression in human cells.

PROTOCOLS

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

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

HIF PHD3 (P-16): sc-46030 is recommended as a control antibody for monitoring of HIF PHD3 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 HIF PHD3 gene expression knockdown using RT-PCR Primer: HIF PHD3 (h)-PR: sc-45799-PR (20 μ l, 479 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.

STORAGE

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

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.