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HIF-1 α shRNA (r) Lentiviral Particles: sc-45919-V

BACKGROUND

Cell growth and viability is compromised by oxygen deprivation (hypoxia). Hypoxia-inducible factors, including HIF-1 α , Arnt 1 (also designated HIF-1 β), EPAS-1 (also designated HIF-2 α) and HIF-3 α , induce glycolysis, erythropoiesis and angiogenesis in order to restore oxygen homeostasis. Hypoxia-inducible factors are members of the Per-Arnt-Sim (PAS) domain transcription factor family. In response to hypoxia, HIF-1 α is upregulated and forms a heterodimer with Arnt 1 to form the HIF-1 complex. The HIF-1 complex recognizes and binds to the hypoxia responsive element (HRE) of hypoxia-inducible genes, thereby activating transcription. Hypoxia-inducible expression of some genes, such as Glut-1, p53, p21 or Bcl-2, is HIF-1 α dependent, whereas expression of others, such as p27, GADD 153 or HO-1, is HIF-1 α independent. EPAS-1 and HIF-3 α have also been shown to form heterodimeric complexes with Arnt 1 in response to hypoxia.

REFERENCES

1. Wang, G.L., et al. 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc. Natl. Acad. Sci. USA* 92: 5510-5514.
2. Tian, H., et al. 1997. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev.* 11: 72-82.
3. Luo, G., et al. 1997. Molecular characterization of the murine HIF-1 α locus. *Gene Expr.* 6: 287-299.
5. Gu, Y.Z., et al. 1998. Molecular characterization and chromosomal localization of a third α -class hypoxia inducible factor subunit, HIF-3 α . *Gene Expr.* 7: 205-213.
6. Wood, S.M., et al. 1998. Selection and analysis of a mutant cell line defective in the hypoxia-inducible factor-1 α -subunit (HIF-1 α). Characterization of HIF-1 α -dependent and -independent hypoxia-inducible gene expression. *J. Biol. Chem.* 273: 8360-8368.

CHROMOSOMAL LOCATION

Genetic locus: Hif1a (rat) mapping to 6q24.

PRODUCT

HIF-1 α shRNA (r) 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 x 10⁶ lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see HIF-1 α siRNA (r): sc-45919 and HIF-1 α shRNA Plasmid (r): sc-45919-SH as alternate gene silencing products.

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.

APPLICATIONS

HIF-1 α shRNA (r) Lentiviral Particles is recommended for the inhibition of HIF-1 α expression in rat cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ 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-1 α (H-206): sc-10790 is recommended as a control antibody for monitoring of HIF-1 α 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz MarkerTM compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz MarkerTM Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruzTM Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HIF-1 α gene expression knockdown using RT-PCR Primer: HIF-1 α (r)-PR: sc-45919-PR (20 μ l, 427 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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