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EPAS-1 siRNA (r): sc-270047

BACKGROUND

Cell growth and viability is compromised by oxygen deprivation (hypoxia). Hypoxia-inducible factors, including HIF-1 α , HIF-1 β (also designated Arnt 1), 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.
4. Carmeliet, P., et al. 1998. Role of HIF-1 α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 394: 485-490.
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: *Epas1* (rat) mapping to 6q12.

PRODUCT

EPAS-1 siRNA (r) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EPAS-1 shRNA Plasmid (r): sc-270047-SH and EPAS-1 shRNA (r) Lentiviral Particles: sc-270047-V as alternate gene silencing products.

For independent verification of EPAS-1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270047A, sc-270047B and sc-270047C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

EPAS-1 siRNA (r) is recommended for the inhibition of EPAS-1 expression in rat cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

EPAS-1 (190b): sc-13596 is recommended as a control antibody for monitoring of EPAS-1 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EPAS-1 gene expression knockdown using RT-PCR Primer: EPAS-1 (r)-PR: sc-270047-PR (20 μ l, 582 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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