

## EPAS-1 siRNA (h): sc-35316

### BACKGROUND

Cell growth and viability is compromised by oxygen deprivation (hypoxia). Hypoxia-inducible factors, including HIF-1 $\alpha$ , HIF-1 $\beta$  (also designated Arnt 1), EPAS-1 (also designated HIF-2 $\alpha$ ) and HIF-3 $\alpha$ , 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 $\alpha$  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 $\alpha$  dependent, whereas expression of others, such as p27, GADD 153 or HO-1, is HIF-1 $\alpha$  independent. EPAS-1 and HIF-3 $\alpha$  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 O<sub>2</sub> tension. *Proc. Natl. Acad. Sci. USA* 92: 5510-5514.
2. Tian, H., et al. 1997. Endothelial PAS domain protein 1 (EPAS-1), a transcription factor selectively expressed in endothelial cells. *Genes Dev.* 11: 72-82.

### CHROMOSOMAL LOCATION

Genetic locus: EPAS1 (human) mapping to 2p21.

### PRODUCT

EPAS-1 siRNA (h) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EPAS-1 shRNA Plasmid (h): sc-35316-SH and EPAS-1 shRNA (h) Lentiviral Particles: sc-35316-V as alternate gene silencing products.

For independent verification of EPAS-1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35316A, sc-35316B and sc-35316C.

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

### APPLICATIONS

EPAS-1 siRNA (h) is recommended for the inhibition of EPAS-1 expression in human 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  $\mu$ M in 66  $\mu$ 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).

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EPAS-1 gene expression knockdown using RT-PCR Primer: EPAS-1 (h)-PR: sc-35316-PR (20  $\mu$ l, 476 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### SELECT PRODUCT CITATIONS

1. Schwalm, S., et al. 2008. Sphingosine kinase-1 is a hypoxia-regulated gene that stimulates migration of human endothelial cells. *Biochem. Biophys. Res. Commun.* 368: 1020-1025.
2. Mandl, M., et al. 2013. Hypoxia-inducible factor-1 $\beta$  (HIF-1 $\beta$ ) is upregulated in a HIF-1 $\alpha$ -dependent manner in 518A2 human melanoma cells under hypoxic conditions. *Biochem. Biophys. Res. Commun.* 434: 166-172.
3. Gomez-Maldonado, L., et al. 2015. EFNA3 long noncoding RNAs induced by hypoxia promote metastatic dissemination. *Oncogene* 34: 2609-2620.
4. Roche, O., et al. 2016. Identification of non-coding genetic variants in samples from hypoxemic respiratory disease patients that affect the transcriptional response to hypoxia. *Nucleic Acids Res.* 44: 9315-9330.
5. Labrousse-Arias, D., et al. 2017. VHL promotes immune response against renal cell carcinoma via NF $\kappa$ B-dependent regulation of VCAM-1. *J. Cell Biol.* 216: 835-847.
6. Yang, S.L., et al. 2018. Hepatitis B virus upregulates GP73 expression by activating the HIF-2 $\alpha$  signaling pathway. *Oncol. Lett.* 15: 5264-5270.
7. Torres, Á., et al. 2019. Extracellular adenosine promotes cell migration/invasion of glioblastoma stem-like cells through A<sub>3</sub> adenosine receptor activation under hypoxia. *Cancer Lett.* 446: 112-122.
8. Colson, A., et al. 2020. Hypoxia-inducible factor 2  $\alpha$  impairs human cytotrophoblast syncytialization: new insights into placental dysfunction and fetal growth restriction. *FASEB J.* 34: 15222-15235.

### RESEARCH USE

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