

## FIH-1 siRNA (m): sc-37886

### BACKGROUND

Factor inhibiting HIF-1 (FIH-1) exists as a homodimer and binds to HIF-1 $\alpha$ . Specifically, FIH-1 operates as an asparaginyl hydroxylase. It catalyzes the hydroxylation of the  $\beta$ -carbon of Asparagine residue 803 within the carboxy terminal transactivation domain of HIF-1 $\alpha$ . This hydroxylation event blocks the association of HIF-1 $\alpha$  with co-activators. FIH-1 also binds to von Hippel-Lindau (VHL) tumor suppressor protein, which represses transcriptional activity of HIF-1 $\alpha$ . In transiently transfected human osteosarcoma cells, FIH-1 localizes to the cytoplasm. The structure of FIH-1 includes a jellyroll-like  $\beta$ -barrel containing ferrous-binding triad residues. The gene encoding human FIH-1 maps to chromosome 10q24.

### REFERENCES

1. Mahon, P.C., et al. 2001. FIH-1: a novel protein that interacts with HIF-1 $\alpha$  and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev.* 15: 2675-2686.
2. Dann, C.E., et al. 2002. Structure of factor-inhibiting hypoxia-inducible factor 1: an asparaginyl hydroxylase involved in the hypoxic response pathway. *Proc. Natl. Acad. Sci. USA* 99: 15351-15356.
3. Lando, D., et al. 2002. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev.* 16: 1466-1471.
4. McNeill, L.A., et al. 2002. Hypoxia-inducible factor asparaginyl hydroxylase (FIH-1) catalyzes hydroxylation at the  $\beta$ -carbon of asparagine-803. *Biochem. J.* 367: 571-575.

### CHROMOSOMAL LOCATION

Genetic locus: Hif1an (mouse) mapping to 19 C3.

### PRODUCT

FIH-1 siRNA (m) 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 FIH-1 shRNA Plasmid (m): sc-37886-SH and FIH-1 shRNA (m) Lentiviral Particles: sc-37886-V as alternate gene silencing products.

For independent verification of FIH-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37886A, sc-37886B and sc-37886C.

### 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

FIH-1 siRNA (m) is recommended for the inhibition of FIH-1 expression in mouse 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

FIH-1 (A-5): sc-271780 is recommended as a control antibody for monitoring of FIH-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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 FIH-1 gene expression knockdown using RT-PCR Primer: FIH-1 (m)-PR: sc-37886-PR (20  $\mu$ l, 571 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### SELECT PRODUCT CITATIONS

1. Zhang, Y., et al. 2021. miR-212 promotes renal interstitial fibrosis by inhibiting hypoxia-inducible factor 1- $\alpha$  inhibitor. *Mol. Med. Rep.* 23: 189.

### RESEARCH USE

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

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.