

Pit-1 siRNA (m): sc-36235

BACKGROUND

Transcriptional regulators play a critical role in development by mediating tissue- and cell-specific transcription. POU domain factors are transcriptional regulators characterized by a bipartite DNA binding domain, which consists of two highly conserved regions, tethered by a variable linker of 14-26 amino acids. Pit-1, also known as growth hormone factor-1 (GHF-1), a member of the POU homeodomain family, is essential for the normal development of the anterior pituitary gland, where it is required for the formation of somatotropes, lactotropes and thyrotropes. In somatotropes and lactotropes, Pit-1 activates the production of growth hormone and Prolactin, respectively. In addition, Pit-1 acts as a repressor of gene expression, which allows for the differentiation of specific cell types. Pit-1 is expressed as two alternatively spliced products, designated Pit-1 α and Pit-1 β , which differ in their *trans*-activation ability. Mutations in the Pit-1 gene are believed to result in combined pituitary hormone deficiency (CPHD) for growth hormone, Prolactin and thyroid stimulating hormone. The gene which encodes Pit-1 maps to human chromosome 3p11.2.

REFERENCES

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- Voss, J.W., et al. 1991. Alternative translation initiation site usage results in two structurally distinct forms of Pit-1. *J. Biol. Chem.* 266: 12832-12835.
- Morris, A.E., et al. 1992. An alternatively spliced Pit-1 isoform altered in its ability to *trans*-activate. *Nucleic Acids Res.* 20: 1355-1361.
- Ohta, K., et al. 1993. Characterization of the gene encoding human pituitary-specific transcription factor, Pit-1. *Gene* 122: 387-388.
- Smith, K.P., et al. 1995. Pit-1 exhibits a unique promoter spacing requirement for activation and synergism. *J. Biol. Chem.* 270: 4484-4491.
- Brown, M.R., et al. 1998. Central hypothyroidism reveals compound heterozygous mutations in the Pit-1 gene. *Horm. Res.* 49: 98-102.
- Schonemann, M.D., et al. 1999. POU domain factors in neural development. *Adv. Exp. Med. Biol.* 449: 39-53.

CHROMOSOMAL LOCATION

Genetic locus: Pou1f1 (mouse) mapping to 16 C1.3.

PRODUCT

Pit-1 siRNA (m) is a pool of 2 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 Pit-1 shRNA Plasmid (m): sc-36235-SH and Pit-1 shRNA (m) Lentiviral Particles: sc-36235-V as alternate gene silencing products.

For independent verification of Pit-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-36235A and sc-36235B.

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

Pit-1 siRNA (m) is recommended for the inhibition of Pit-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 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

Pit-1 (G-2): sc-25258 is recommended as a control antibody for monitoring of Pit-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 Pit-1 gene expression knockdown using RT-PCR Primer: Pit-1 (m)-PR: sc-36235-PR (20 μ l, 432 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.