

Six2 siRNA (m): sc-38787

BACKGROUND

The Six proteins (sine oculis) are a family of homeodomain transcription factors that share a conserved DNA binding domain. Six2, Six4 (AREC3) and Six5 bind to the same DNA sequence, indicating that they may regulate the same target genes. Six1 and Six4 are both capable of transactivating MEF3 site containing reporter genes, such as myogenin. It has been demonstrated that alterations to homeobox-containing genes may result in cancer. Six1 expression has been shown to be absent or low in normal adult tissues, although it is expressed in several tumor types, including breast carcinoma. Six1 overexpression has been shown to abrogate the G₂ cell cycle checkpoint. Six2 is highly expressed in fetal tissues but expression is limited in adult tissues.

REFERENCES

1. Cillo, C. 1994. HOX genes in human cancers. *Invasion Metastasis* 14: 38-49.
2. Paules, R.S., et al. 1995. Defective G₂ checkpoint function in cells from individuals with familial cancer syndromes. *Cancer Res.* 55: 1763-1773.
3. Kawakami, K., et al. 1996. Identification and expression of Six family genes in mouse retina. *FEBS Lett.* 393: 259-263.
4. Davey, S., et al. 1998. Fission yeast rad12⁺ regulates cell cycle checkpoint control and is homologous to the Bloom's syndrome disease gene. *Mol. Cell. Biol.* 18: 2721-2728.
5. Ford, H.L., et al. 1998. Abrogation of the G₂ cell cycle checkpoint associated with overexpression of HSix1: a possible mechanism of breast carcinogenesis. *Proc. Natl. Acad. Sci. USA* 95: 12608-12613.
6. Spitz, F., et al. 1998. Expression of myogenin during embryogenesis is controlled by Six/sine oculis homeoproteins through a conserved MEF3 binding site. *Proc. Natl. Acad. Sci. USA* 95: 14220-14225.

CHROMOSOMAL LOCATION

Genetic locus: Six2 (mouse) mapping to 17 E4.

PRODUCT

Six2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Six2 shRNA Plasmid (m): sc-38787-SH and Six2 shRNA (m) Lentiviral Particles: sc-38787-V as alternate gene silencing products.

For independent verification of Six2 (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-38787A, sc-38787B and sc-38787C.

PROTOCOLS

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

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

Six2 siRNA (m) is recommended for the inhibition of Six 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

Six2 (H-4): sc-377193 is recommended as a control antibody for monitoring of Six2 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 Six2 gene expression knockdown using RT-PCR Primer: Six2 (m)-PR: sc-38787-PR (20 μ l, 408 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.