

Midline-1 siRNA (h): sc-62614

BACKGROUND

Midline-1 (tripartite motif-containing protein 18, putative transcription factor XPRF, RING finger protein 59) is a 667 amino acid protein encoded by the human gene MID1. Midline-1 belongs to the TRIM/RBCC family and contains two B box-type zinc fingers, one B30.2/SPRY domain, one COS domain, one fibronectin type-III domain and one RING-type zinc finger. Midline-1 is believed to have E3 ubiquitin ligase activity which targets the catalytic subunit of protein phosphatase 2 for degradation. It is a cytoplasmic protein found as a homodimer or heterodimer with Midline-2. It also interacts with IGBP1 (lymphocyte signaling protein A4). Defects in MID1 are the cause of Opitz syndrome type I (OS-I). OS-I is an X-linked recessive disorder characterized by hypertelorism, genital-urinary defects such as hypospadias in males and splayed labia in females, lip-palate-laryngotracheal clefts, imperforate anus, developmental delay and congenital heart defects. OS-I mutations produce proteins with a decreased affinity for microtubules.

REFERENCES

1. Gaudenz, K., et al. 1998. Opitz G/BBB syndrome in Xp22: mutations in the MID1 gene cluster in the carboxy-terminal domain. *Am. J. Hum. Genet.* 63: 703-710.
2. Buchner, G., et al. 1999. MID2, a homologue of the Opitz syndrome gene MID1: similarities in subcellular localization and differences in expression during development. *Hum. Mol. Genet.* 8: 1397-1407.
3. Schweiger, S., et al. 1999. The Opitz syndrome gene product, MID1, associates with microtubules. *Proc. Natl. Acad. Sci. USA* 96: 2794-2799.
4. Trockenbacher, A., et al. 2001. MID1, mutated in Opitz syndrome, encodes an ubiquitin ligase that targets phosphatase 2A for degradation. *Nat. Genet.* 29: 287-294.
5. Short, K.M., et al. 2002. MID1 and MID2 homo- and heterodimerise to tether the rapamycin-sensitive PP2A regulatory subunit, Alpha 4, to microtubules: implications for the clinical variability of X-linked Opitz GBBB syndrome and other developmental disorders. *BMC Cell Biol.* 3: 1.

CHROMOSOMAL LOCATION

Genetic locus: MID1 (human) mapping to Xp22.2.

PRODUCT

Midline-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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Midline-1 shRNA Plasmid (h): sc-62614-SH and Midline-1 shRNA (h) Lentiviral Particles: sc-62614-V as alternate gene silencing products.

For independent verification of Midline-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-62614A, sc-62614B and sc-62614C.

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

Midline-1 siRNA (h) is recommended for the inhibition of Midline-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 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

Midline-1 (2C11): sc-293353 is recommended as a control antibody for monitoring of Midline-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 Midline-1 gene expression knockdown using RT-PCR Primer: Midline-1 (h)-PR: sc-62614-PR (20 μ l). 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.