

WTAP siRNA (m): sc-63225

BACKGROUND

Wilms' tumor (WT) is an embryonal malignancy of the kidney that affects 1 in 10,000 infants and is observed in both sporadic and inherited forms. The Wilms' tumor protein (WT1) binds the DNA sequence GCGGGGCG, a recognition element common to the early growth response (Egr) family of Zn²⁺ finger transcriptional activators, and functions as a transcriptional repressor. WTAP (Wilms' tumor 1-associating protein) is a ubiquitously expressed nuclear protein that interacts with WT1 and may be involved in regulating mRNA splicing. WTAP is found in nuclear speckles where it regulates the G₂/M cell cycle transition by binding to the 3' UTR of cyclin A2, thus enhancing its stability. Additionally, WTAP inhibits expression of WT1 target genes and is able to impair the ability of WT1 to bind DNA. Two isoforms of WTAP exist due to alternative splicing events.

REFERENCES

1. Branzei, D., et al. 2001. A novel protein interacts with the Werner's syndrome gene product physically and functionally. *J. Biol. Chem.* 276: 20364-20369.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605442. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Utsch, B., et al. 2003. Exclusion of WTAP and HOXA13 as candidate genes for isolated hypospadias. *Scand. J. Urol. Nephrol.* 37: 498-501.
4. Chen, B.F., et al. 2004. Immunohistochemical expression of Wilms' tumor 1 protein in nephroblastoma. *J. Chin. Med. Assoc.* 67: 506-510.
5. Horiuchi, K., et al. 2006. Wilms' tumor 1-associating protein regulates G₂/M transition through stabilization of cyclin A2 mRNA. *Proc. Natl. Acad. Sci. USA* 103: 17278-17283.
6. Rong, Y., et al. 2006. Wilms' tumor 1 and signal transducers and activators of transcription 3 synergistically promote cell proliferation: a possible mechanism in sporadic Wilms' tumor. *Cancer Res.* 66: 8049-8057.

CHROMOSOMAL LOCATION

Genetic locus: Wtap (mouse) mapping to 17 A1.

PRODUCT

WTAP 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see WTAP shRNA Plasmid (m): sc-63225-SH and WTAP shRNA (m) Lentiviral Particles: sc-63225-V as alternate gene silencing products.

For independent verification of WTAP (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-63225A, sc-63225B and sc-63225C.

RESEARCH USE

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

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 µl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 µl of RNase-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

WTAP siRNA (m) is recommended for the inhibition of WTAP 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 µM in 66 µl. 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

WTAP (D-7): sc-374280 is recommended as a control antibody for monitoring of WTAP 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 WTAP gene expression knockdown using RT-PCR Primer: WTAP (m)-PR: sc-63225-PR (20 µl, 334 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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