SANTA CRUZ BIOTECHNOLOGY, INC.

VASA siRNA (h): sc-61772

BACKGROUND

VASA is a 724 amino acid, ATP-dependent RNA helicase that belongs to the DEAD-box family. VASA is specifically expressed in germline cells throughout the life cycle and is undetectable in somatic tissues. In vertebrates, VASA is restricted to bisexually reproducing organisms. It is cytoplasmic and is present only in migratory primordial germ cells in the region of the gonadal ridge. On testicular sections, VASA expression is the highest in spermatogonia, reduced in spermatocytes, low in spermatids and absent in sperm. In the ovary, VASA expression is the highest in oogonia but persists throughout oogenesis. VASA has a glycine-rich N-terminus with multiple repeats of an RGG motif believed to function in RNA binding. Specifically, it regulates the translation of intricate mRNAs that are essential for differentiation.

REFERENCES

1. Castrillon, D.H., et al. 2000. The human VASA gene is specifically expressed in the germ cell lineage. Proc. Natl. Acad. Sci. USA 97: 9585-9590.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605281. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Honecker, F., et al. 2004. Pathobiological implications of the expression of markers of testicular carcinoma *in situ* by fetal germ cells. J. Pathol. 203: 849-857.
4. Pennetier, S., et al. 2004. Spatio-temporal expression of the germ cell marker genes MATER, ZAR1, GDF9, BMP15 and VASA in adult bovine tissues, oocytes, and preimplantation embryos. Biol. Reprod. 71: 1359-1366.
5. Abdelhaleem, M. 2005. RNA helicases: regulators of differentiation. Clin. Biochem. 38: 499-503.
6. Stoop, H., et al. 2005. Differentiation and development of human female germ cells during prenatal gonadogenesis: an immunohistochemical study. Hum. Reprod. 20: 1466-1476.
7. Xu, H., et al. 2005. Differential expression of VASA RNA and protein during spermatogenesis and oogenesis in the gibel carp *(Carassius auratus gibelio),* a bisexually and gynogenetically reproducing vertebrate. Dev. Dyn. 233: 872-882.

CHROMOSOMAL LOCATION

Genetic locus: DDX4 (human) mapping to 5q11.2.

PRODUCT

VASA 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see VASA shRNA Plasmid (h): sc-61772-SH and VASA shRNA (h) Lentiviral Particles: sc-61772-V as alternate gene silencing products.

For independent verification of VASA (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-61772A, sc-61772B and sc-61772C.

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

VASA siRNA (h) is recommended for the inhibition of VASA 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 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

VASA (2F9H5): sc-517247 is recommended as a control antibody for monitoring of VASA 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 VASA gene expression knock down using RT-PCR Primer: VASA (h)-PR: sc-61772-PR (20 µl, 462 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.