SANTA CRUZ BIOTECHNOLOGY, INC.

OVCA1 siRNA (h): sc-62727

BACKGROUND

OVCA1 (ovarian cancer-associated gene 1 protein) is a nuclear protein that belongs to the DPH1/DPH2 family (DPH1 subfamily). Diphthamide is a modified histidine amino acid that is created by a posttranslational modification of eukaryotic elongation factor 2 (EF-2). Diphthamide is the target for ADP-ribosylation by diphtheria toxin, which renders the elongation factor inactive. The diphthamide modification is conserved in all eukaryotes and archaebacteria and is formed by the actions of five proteins, DPH1-5, and an amidating enzyme. OVCA1 (DPH1) is required for the first step in the synthesis of diphthamide. The chromosomal region that contains OVCA1, 17p13.3, is frequently deleted in human ovarian carcinoma, suggesting this 15 kb region of deletion may contain a tumor suppressor gene. OVCA1 acts as a tumor suppressor in lung and breast cancers and when overexpressed will suppress colony formation and growth rate of ovarian cancer cells. OVCA1 is expressed in heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, mammary gland, colon, small intestine, testis and ovary.

REFERENCES

1. Phillips, N.J., et al. 1996. A cDNA from the ovarian cancer critical region of deletion on chromosome 17p13.3. Cancer Lett. 102: 85-90.
2. Schultz, D.C., et al. 1996. Identification of two candidate tumor suppressor genes on chromosome 17p13.3. Cancer Res. 56: 1997-2002.
3. Salicioni, A.M., et al. 2000. Identification and structural analysis of human RBM8A and RBM8B: two highly conserved RNA-binding motif proteins that interact with OVCA1, a candidate tumor suppressor. Genomics 69: 54-62.
4. Jensen, M.R. and Helin, K. 2004. OVCA1: emerging as a bona fide tumor suppressor. Genes Dev. 18: 245-248.
5. Nobukuni, Y., et al. 2005. Gene trap mutagenesis-based forward genetic approach reveals that the tumor suppressor OVCA1 is a component of the biosynthetic pathway of diphthamide on elongation factor 2. J. Biol. Chem. 280: 10572-10577.
6. Chen, C.M. and Behringer, R.R. 2005. OVCA.tumor suppressor gene. Curr. Opin. Genet. Dev. 15: 49-54.

CHROMOSOMAL LOCATION

Genetic locus: DPH1 (human) mapping to 17p13.3.

PRODUCT

OVCA1 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 OVCA1 shRNA Plasmid (h): sc-62727-SH and OVCA1 shRNA (h) Lentiviral Particles: sc-62727-V as alternate gene silencing products.

For independent verification of OVCA1 (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-62727A, sc-62727B and sc-62727C.

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

OVCA1 siRNA (h) is recommended for the inhibition of OVCA1 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

OVCA1 (6E93): sc-101248 is recommended as a control antibody for monitoring of OVCA1 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 OVCA1 gene expression knockdown using RT-PCR Primer: OVCA1 (h)-PR: sc-62727-PR (20 µl). 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.