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HE4 siRNA (h): sc-43826

BACKGROUND

HE4 (whey acidic protein (WAP)-type four-disulfide core-2, WFDC2) is a small secretory protein that may influence sperm maturation. HE4 gene expression is high in pulmonary epithelial cells and in some ovarian cancers. HE4 protein has a WAP motif that contains eight cysteines forming four disulfide bonds at the core of the protein. The WAP motif functions as a protease inhibitor in many of the family members that contain them.

REFERENCES

1. Kirchoff, C., et al. 1991. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. *Biol. Reprod.* 45: 350-357.
2. Bingle, L., et al. 2002. The putative ovarian tumour marker gene HE4 (WFDC2), is expressed in normal tissues and undergoes complex alternative splicing to yield multiple protein isoforms. *Oncogene* 21: 2768-2773.
3. Hellstrom, I., et al. 2003. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res.* 63: 3695-3700.
4. Hagiwara, K., et al. 2003. Mouse SWAM1 and SWAM2 are antibacterial proteins composed of a single whey acidic protein motif. *J. Immunol.* 170: 1973-1979.
5. Urban, N., et al. 2003. Ovarian cancer screening. *Hematol. Oncol. Clin. North Am.* 17: 989-1005.
6. Berry, N.B., et al. 2004. Transcriptional targeting in ovarian cancer cells using the human epididymis protein 4 promoter. *Gynecol. Oncol.* 92: 896-904.

CHROMOSOMAL LOCATION

Genetic locus: WFDC2 (human) mapping to 20q13.12.

PRODUCT

HE4 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 HE4 shRNA Plasmid (h): sc-43826-SH and HE4 shRNA (h) Lentiviral Particles: sc-43826-V as alternate gene silencing products.

For independent verification of HE4 (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-43826A, sc-43826B and sc-43826C.

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

HE4 siRNA (h) is recommended for the inhibition of HE4 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

HE4 (3F9): sc-293473 is recommended as a control antibody for monitoring of HE4 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 HE4 gene expression knockdown using RT-PCR Primer: HE4 (h)-PR: sc-43826-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.