SANTA CRUZ BIOTECHNOLOGY, INC.

AFP siRNA (m): sc-29649

BACKGROUND

α-fetoprotein (AFP) is expressed in fetal liver at varying levels throughout development and is present only in trace amounts in normal adult tissues. AFP can be detected at abnormally high concentrations in hepatocellular carcinomas as well as in the plasma and ascitic fluid of adults with hepatoma. High AFP concentrations have been correlated with tumor cell growth, indicating that AFP can serve as a tumor marker. AFP binds copper, nickel and fatty acids, and in some cases may bind serum albumin or estrogen. It has been demonstrated that the AFP promoter is a target for NF-1 (nuclear factor-1), HNF-1 (hepatocyte nuclear factor-1) and C/EBP transcription factors. While HNF-1 binding to the AFP promoter results in AFP expression, NF-1 binding results in a decrease in AFP promoter activity.

REFERENCES

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3. Iturralde, M., Alava, M.A., Gonzalez, B., Anel, A. and Pineiro, A. 1991. Effect of α-fetoprotein and albumin on the uptake of polyunsaturated fatty acids by rat hepatoma cells and fetal rat hepatocytes. Biochim. Biophys. Acta 1086: 81-88.
4. Bois-Joyeux, B. and Danan, J.L. 1994. Members of the CAAT/enhancer-binding protein, hepatocyte nuclear factor-1 and nuclear factor-1 families can differentially modulate the activities of the rat α-fetoprotein promoter and enhancer. Biochem. J. 301: 49-55.
5. Ido, A., Nakata, K., Kato, Y., Murata, K., Fujita, M., Ishii, N., Tamaoki, T., Shiku, H. and Nagataki, S. 1995. Gene therapy for hepatoma cells using a retrovirus vector carrying herpes simplex virus thymidine kinase gene under the control of human α-fetoprotein gene promoter. Cancer Res. 55: 3105-3109.

CHROMOSOMAL LOCATION

Genetic locus: Afp (mouse) mapping to 5 E1.

PRODUCT

AFP 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 AFP shRNA Plasmid (m): sc-29649-SH and AFP shRNA (m) Lentiviral Particles: sc-29649-V as alternate gene silencing products.

For independent verification of AFP (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-29649A, sc-29649B and sc-29649C.

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

AFP siRNA (m) is recommended for the inhibition of AFP 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

AFP (C3): sc-8399 is recommended as a control antibody for monitoring of AFP 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 AFP gene expression knockdown using RT-PCR Primer: AFP (m)-PR: sc-29649-PR (20 µl, 567 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.