SANTA CRUZ BIOTECHNOLOGY, INC.

TFPI siRNA (m): sc-41061

BACKGROUND

The extrinsic pathway of blood coagulation is initiated by contact of plasma factor VII with tissue factor, a cellular membrane glycoprotein that normally is segregated from the bloodstream but can be exposed after tissue injury or newly synthesized in endothelial cells or leukocytes after stimulation by endotoxin and cytokines. Inhibition of Factor VIIa tissue factor activity requires a plasma component (tissue factor pathway inhibitor (TFPI), lipoprotein-associated coagulation inhibitor (LACI) or extrinsic pathway inhibitor (EPI)) and factor Xa. TFPI directly inhibits factor Xa, and, in an Xa-dependent fashion, also inhibits the Factor VIIa tissue factor catalytic complex. TFPI is a multivalent, Kunitz-type proteinase inhibitor that circulates in association with plasma lipoproteins VLDL, LDL, and HDL. TFPI-2 (also known as placental protein 5) is a related glycoprotein that was originally isolated from human placenta.

REFERENCES

1. Broze, G.J., Jr., et al. 1987. Characterization of the inhibition of tissue factor in serum. Blood 69: 150-155.
2. Rao, L.V., et al. 1987. Studies of a mechanism inhibiting the initiation of the extrinsic pathway of coagulation. Blood 69: 645-651.
3. Davie, E.W., et al. 1991. The coagulation cascade: initiation, maintenance, and regulation. Biochemistry 30: 10363-10370.
4. Girard, T.J., et al. 1991. Structure of the human lipoprotein-associated coagulation inhibitor gene. Intro/exon gene organization and localization of the gene to chromosome 2. J. Biol. Chem. 266: 5036-5041.
5. Enjyoji, K., et al. 1993. Human tissue factor pathway inhibitor (TFPI) gene: complete genomic structure and localization on the genetic map of chromosome 2q. Genomics 17: 423-428.
6. Kisiel, W., et al. 1994. Evidence that a second human tissue factor pathway inhibitor (TFPI-2) and human placental protein 5 are equivalent. Blood 84: 4384-4385.
7. Miyagi, Y., et al. 1996. Assignment of the human PP5/TFPI-2 gene to 7q22 by FISH and PCR-based human/rodent cell hybrid mapping panel analysis. Genomics 35: 267-268.

CHROMOSOMAL LOCATION

Genetic locus: Tfpi (mouse) mapping to 2 D.

PRODUCT

TFPI 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 TFPI shRNA Plasmid (m): sc-41061-SH and TFPI shRNA (m) Lentiviral Particles: sc-41061-V as alternate gene silencing products.

For independent verification of TFPI (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-41061A, sc-41061B and sc-41061C.

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

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

TFPI (G-5): sc-365920 is recommended as a control antibody for monitoring of TFPI 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 TFPI gene expression knockdown using RT-PCR Primer: TFPI (m)-PR: sc-41061-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.