

TF siRNA (m): sc-40415

BACKGROUND

Hemostasis following tissue injury involves the deployment of essential plasma procoagulants (Prothrombin and Factors X, IX, V and VIII), which are involved in a blood coagulation cascade leading to the formation of insoluble fibrin clots and the promotion of platelet aggregation. Coagulation Factor V (Factor V, FV, proaccelerin, labile factor) is a 2196 amino acid, single chain glycoprotein that is cleaved by Thrombin to yield an active, Ca^{2+} -dependent dimer. This dimer consists of 105 kDa and 74 kDa fragments, and is essential to the blood coagulation cascade. Together with catalytic Factor Xa and Ca^{2+} on the surface of platelets or endothelial cells, Factor Va coordinates into a Prothrombinase complex, which mediates proteolysis of Prothrombin into active Thrombin. Tissue factor (TF, coagulation factor III) is a cell surface glycoprotein that enables cells to initiate blood coagulation cascades, and it functions as a high-affinity receptor for coagulation Factor VII.

REFERENCES

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3. Jenny, R.J., et al. 1987. Complete cDNA and derived amino acid sequence of human Factor V. *Proc. Natl. Acad. Sci. USA* 84: 4846-4850.
4. Davie, E.W., et al. 1991. The coagulation cascade: initiation, maintenance and regulation. *Biochemistry* 30: 10363-10370.
5. Rand, M.D., et al. 1994. Platelet coagulation Factor Va: the major secretory platelet phosphoprotein. *Blood* 83: 2180-2190.
6. Macedo-Ribeiro, S., et al. 1999. Crystal structures of the membrane-binding C2 domain of human coagulation Factor V. *Nature* 402: 434-439.
7. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 227400. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
8. LocusLink Report (LocusID: 2152). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: F3 (mouse) mapping to 3 G1.

PRODUCT

TF 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 TF shRNA Plasmid (m): sc-40415-SH and TF shRNA (m) Lentiviral Particles: sc-40415-V as alternate gene silencing products.

For independent verification of TF (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-40415A, sc-40415B and sc-40415C.

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

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

TF (H-9): sc-374441 is recommended as a control antibody for monitoring of TF 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 TF gene expression knockdown using RT-PCR Primer: TF (m)-PR: sc-40415-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.