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TF shRNA (h) Lentiviral Particles: sc-44984-V

BACKGROUND

Homeostasis 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, Ca²⁺-dependent dimer that is essential to the blood coagulation cascade. Together with catalytic Factor XA and Ca²⁺ 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), also designated coagulation Factor III) is a cell surface glycoprotein that enables cells to initiate blood coagulation cascades. It functions as a high-affinity receptor for coagulation Factor VII.

REFERENCES

1. Davie, E.W., et al. 1975. Basic mechanisms in blood coagulation. *Annu. Rev. Biochem.* 44: 799-829.
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4. Davie, E.W., et al. 1991. The coagulation cascade: initiation, maintenance and regulation. *Biochemistry* 30: 10363-10370.
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CHROMOSOMAL LOCATION

Genetic locus: F3 (human) mapping to 1p21.3.

PRODUCT

TF shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see TF siRNA (h): sc-44984 and TF shRNA Plasmid (h): sc-44984-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

TF shRNA (h) Lentiviral Particles is recommended for the inhibition of TF expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

TF (C-7): sc-393657 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TF gene expression knockdown using RT-PCR Primer: TF (h)-PR: sc-44984-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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