

tPA siRNA (h): sc-36705

BACKGROUND

uPA (urokinase-type plasminogen activator) and tPA (tissue plasminogen activator) are serine proteases that are members of the trypsin family, and they are essential to the intrinsic coagulation system. tPA is primarily involved in fibrinolysis whereas uPA principally mediates cell migration and tissue remodeling processes. uPA and tPA are responsible for cleaving plasminogen, a large serum β -globulin that is deposited on the Fibrin strands within a thrombus. uPA and tPA preferentially target plasminogen at the Arg-Val bond to produce plasmin (also designated fibrinolysin), which is a trypsin-like enzyme that acts on Arg-Lys bonds in Fibrin and Fibrinogen and contributes to the systematic activation of the coagulation cascade. uPA and tPA each consist of two chains that are designated A and B. The A chain of uPA can be cleaved, resulting in low and high molecular mass forms. uPA and tPA are regulated by the serpin family members, PAI-1 and PAI-2, which are serine proteinase inhibitors that complex with uPA, tPA and other targeted proteinases and then slowly disassociate to produce cleaved species that fold into stable inactive conformations.

REFERENCES

1. Riccio, A., et al. 1985. The human urokinase-plasminogen activator gene and its promoter. *Nucleic Acids Res.* 13: 2759-2771.
2. Degen, S.J., et al. 1986. The human tissue plasminogen activator gene. *J. Biol. Chem.* 261: 6972-6985.
3. Milligan, K.S. 1987. Tissue-type plasminogen activator: a new fibrinolytic agent. *Heart Lung* 16: 69-74.
4. Loscalzo, J., et al. 1988. Tissue plasminogen activator. *N. Engl. J. Med.* 319: 925-931.

CHROMOSOMAL LOCATION

Genetic locus: PLAT (human) mapping to 8p11.21.

PRODUCT

tPA 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 tPA shRNA Plasmid (h): sc-36705-SH and tPA shRNA (h) Lentiviral Particles: sc-36705-V as alternate gene silencing products.

For independent verification of tPA (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-36705A, sc-36705B and sc-36705C.

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

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

tPA (D-1): sc-515562 is recommended as a control antibody for monitoring of tPA 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 tPA gene expression knockdown using RT-PCR Primer: tPA (h)-PR: sc-36705-PR (20 μ l, 478 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Montt-Guevara, M.M., et al. 2017. Regulatory effects of estetrol on the endothelial plasminogen pathway and endothelial cell migration. *Maturitas* 99: 1-9.

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.