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- Trockeneiszuschlag
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- Expressversand

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# tPA shRNA (r) Lentiviral Particles: sc-45948-V

## BACKGROUND

uPA (urokinase-type plasminogen activator) and tPA (tissue plasminogen activator), which are serine proteases and members of the trypsin family, 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  $\beta$ -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. Cheng, X.F., et al. 1992. Binding of tissue plasminogen activator to human endothelial cells. Importance of the B-chain as a ligand. *Biochem. J.* 287: 407-413.

## CHROMOSOMAL LOCATION

Genetic locus: Plat (rat) mapping to 16q12.5.

## PRODUCT

tPA shRNA (r) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 tPA siRNA (r): sc-45948 and tPA shRNA Plasmid (r): sc-45948-SH as alternate gene silencing products.

## STORAGE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

tPA shRNA (r) Lentiviral Particles is recommended for the inhibition of tPA expression in rat cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

tPA (H-90): sc-15346 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor tPA gene expression knockdown using RT-PCR Primer: tPA (r)-PR: sc-45948-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## 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.