SANTA CRUZ BIOTECHNOLOGY, INC.

TM siRNA (m): sc-36687

BACKGROUND

Thrombomodulin™, also called CD141, is a type I membrane receptor that is specific to endothelial cells. TM has a cysteine-rich extracellular domain with six EGF-like regions. It forms a complex with Thrombin, which activates Protein C to generate activated Protein C (APC), an anticoagulant enzyme. APC together with Protein S inhibits coagulation by inactivating Factors Va and VIIIa. Deletion of the TM gene results in embryonic lethality in mice.

REFERENCES

1. Jackman, R.W., et al. 1987. Human thrombomodulin gene is intron depleted: nucleic acid sequences of the cDNA and gene predict protein structure and suggest sites of regulatory control. Proc. Natl. Acad. Sci. USA 84: 6425-6429.
2. Suzuki, K., et al. 1987. Structure and expression of human thrombomodulin, a thrombin receptor on endothelium acting as a cofactor for Protein C activation. EMBO J. 6: 1891-1897.
3. Shirai, T., et al. 1988. Gene structure of human thrombomodulin, a cofactor for thrombin-catalyzed activation of Protein C. J. Biochem. 103: 281-285.
4. Healy, A.M., et al. 1995. Absence of the blood-clotting regulator thrombomodulin causes embryonic lethality in mice before development of a functional cardiovascular system. Proc. Natl. Acad. Sci. USA 92: 850-854.
5. Rosenberg, R.D. 1995. The absence of the blood clotting regulator thrombomodulin causes embryonic lethality in mice before development of a functional cardiovascular system. Thromb. Haemost. 74: 52-57.
6. Nishioka, J., et al. 1996. The Gla26 residue of Protein C is required for the binding of Protein C to thrombomodulin and endothelial cell Protein C receptor, but not to Protein S and Factor Va. Thromb. Haemost. 75: 275-282.
7. Gerlitz, B., et al. 1996. Mutation of protease domain residues Ly37-39 in human Protein C inhibits activation by the thrombomodulin-thrombin complex without affecting activation by free thrombin. J. Biol. Chem. 271: 22285-22288.
8. Dahlback, B. 1997. Factor V and Protein S as cofactors to activated Protein C. Haematologica 82: 91-95.

CHROMOSOMAL LOCATION

Genetic locus: Thbd (mouse) mapping to 2 G3.

PRODUCT

TM 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 TM shRNA Plasmid (m): sc-36687-SH and TM shRNA (m) Lentiviral Particles: sc-36687-V as alternate gene silencing products.

For independent verification of TM (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-36687A, sc-36687B and sc-36687C.

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

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

TM (H-11): sc-271804 is recommended as a control antibody for monitoring of TM 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 TM gene expression knockdown using RT-PCR Primer: TM (m)-PR: sc-36687-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.