SANTA CRUZ BIOTECHNOLOGY, INC.

Prothrombin siRNA (m): sc-40414

BACKGROUND

Hemostasis following tissue injury involves the deployment of essential plasma procoagulants (Prothrombin and Factors X, IX, V and VIII), which mediate a blood coagulation cascade that leads to the formation of insoluble Fibrin clots and the promotion of platelet aggregation. Proteolytic cleavage of Prothrombin (Factor II) at residue 44 leads to formation of Thrombin in the first step of the coagulation cascade. Thrombin cleaves bonds between Arg and Gly and activates Factors V, VII, VIII and XIII in complex with thrombomodulin and Protein C. Thrombin maintains vascular integrity during development and postnatal life and coordinates connective tissue proteins by stimulating fibroblast procollagen production.

REFERENCES

1. Davey, M.G., et al. 1967. Actions of Thrombin and other coagulant and proteolytic enzymes on blood platelets. Nature 216: 857-858.
2. Davie, E.W., et al. 1975. Basic mechanisms in blood coagulation. Annu. Rev. Biochem. 44: 799-829.
3. Elion, J., et al. 1986. Proteolytic derivatives of Thrombin. Ann. N.Y. Acad. Sci. 485: 16-26.
4. Royle, N.J., et al. 1987. Human genes encoding prothrombin and ceruloplasmin map to 11p11-q12 and 3q21-24, respectively. Somat. Cell Mol. Genet. 13: 285-292.
5. Davie, E.W., et al. 1991. The coagulation cascade: initiation, maintenance, and regulation. Biochemistry 30: 10363-10370.
6. Chambers, R.C., et al. 1998. Thrombin stimulates fibroblast procollagen production via proteolytic activation of protease-activated receptor 1. Biochem. J. 333: 121-127.
7. Huang, Y.Q., et al. 2000. Thrombin inhibits tumor cell growth in association with up-regulation of p21(waf/CIP1) and caspases via a p53-independent, Stat1-dependent pathway. J. Biol. Chem. 275: 6462-6468.
8. LocusLink Report (LocusID: 2147). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: F2 (mouse) mapping to 2 E1.

PRODUCT

Prothrombin 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 Prothrombin shRNA Plasmid (m): sc-40414-SH and Prothrombin shRNA (m) Lentiviral Particles: sc-40414-V as alternate gene silencing products.

For independent verification of Prothrombin (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-40414A, sc-40414B and sc-40414C.

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

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

Thrombin (F-1): sc-271449 is recommended as a control antibody for monitoring of Thrombin 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 Prothrombin gene expression knockdown using RT-PCR Primer: Prothrombin (m)-PR: sc-40414-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.