

Protein S siRNA (h): sc-63328

BACKGROUND

Protein S (PROS) is a vitamin K-dependent plasma protein that inhibits blood clotting by serving as a cofactor for activated protein C (APC) and facilitates clearance of early apoptotic cells. In the plasma, circulating Protein S becomes inactive upon complexing with C4b-binding protein (C4BP); 60-70% of Protein S circulates in complex with C4BP. Calcium-dependent association of C4BP-Protein S with apoptotic cells influences the regulation of complement activation. Protein S has APC-independent anticoagulant activity through direct inhibition of prothrombin activation via interactions with Factor X A, Factor V A and phospholipids. Autosomal dominant Protein S deficiency (levels 15 to 37% of normal) correlates with severe recurrent venous thrombosis.

REFERENCES

1. Stenflo, J., et al. 1979. Protein S, a new vitamin K-dependent protein from bovine plasma. *FEBS Lett.* 101: 377-381.
2. DiScipio, R.G., et al. 1979. Characterization of Protein S, a γ -carboxyglutamic acid containing protein from bovine and human plasma. *Biochemistry* 18: 899-904.
3. Dahlback, B., et al. 1981. High molecular weight complex in human plasma between vitamin K-dependent Protein S and complement component C4b-binding protein. *Proc. Nat. Acad. Sci. USA* 78: 2512-2516.
4. Comp, P.C., et al. 1984. Recurrent venous thromboembolism in patients with a partial deficiency of Protein S. *N. Engl. J. Med.* 311: 1525-1528.
5. Broekmans, A.W., et al. 1985. Hereditary Protein S deficiency and venous thrombo-embolism. A study in three Dutch families. *Thromb. Haemost.* 53: 273-277.
6. Andersen, B.D., et al. 2001. Characterization and structural impact of five novel PROS1 mutations in eleven Protein S-deficient families. *Thromb. Haemost.* 86: 1392-1399.
7. Sere, K.M., et al. 2001. Purified Protein S contains multimeric forms with increased APC-independent anticoagulant activity. *Biochemistry* 40: 8852-8860.

CHROMOSOMAL LOCATION

Genetic locus: PROS1 (human) mapping to 3q11.1.

PRODUCT

Protein S 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 Protein S shRNA Plasmid (h): sc-63328-SH and Protein S shRNA (h) Lentiviral Particles: sc-63328-V as alternate gene silencing products.

For independent verification of Protein S (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-63328A, sc-63328B and sc-63328C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Protein S siRNA (h) is recommended for the inhibition of Protein S 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

Protein S (F-10): sc-271326 is recommended as a control antibody for monitoring of Protein S 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 Protein S gene expression knockdown using RT-PCR Primer: Protein S (h)-PR: sc-63328-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.