

# Factor X siRNA (h): sc-40405

## BACKGROUND

Hemostasis following tissue injury involves the deployment of essential plasma procoagulants (prothrombin, and factors X, IX, V and VIII), which are involved in a blood coagulation cascade that leads to the formation of insoluble fibrin clots and the promotion of platelet aggregation. Coagulation factor X (Stuart Prower factor, FX, F10) is a vitamin K-dependent, single chain serine protease that is synthesized in the liver and circulates as an inactive precursor. The mature form of FX (FXa) is generated by factor IXa or factor VIIa mediated cleavage at the tripeptide sequence, Arg-Lys-Arg, to yield a disulfide linked dimer. Together with the cofactor FVa, and Ca<sup>2+</sup> on the surface of platelets or endothelial cells, Factor Xa coordinates as part of the prothrombinase complex, which mediates proteolysis of prothrombin into active thrombin. Mutations at the FX locus resulting in factor X deficiencies can contribute to hemorrhagic diathesis.

## REFERENCES

1. Davie, E.W. and Fujikawa, K. 1975. Basic mechanisms in blood coagulation. *Annu. Rev. Biochem.* 44: 799-829.
2. Di Scipio, R.G., et al. 1977. A comparison of human prothrombin, Factor IX (Christmas factor), Factor X (Stuart factor), and protein S. *Biochemistry* 16: 698-706.
3. Davie, E.W., et al. 1991. The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry* 30: 10363-10370.
4. Macedo-Ribeiro, S., et al. 1999. Crystal structures of the membrane-binding C2 domain of human coagulation Factor V. *Nature* 402: 434-439.
5. Chambers, R.C., et al. 2000. Thrombin is a potent inducer of connective tissue growth factor production via proteolytic activation of protease-activated receptor-1. *J. Biol. Chem.* 275: 35584-35591.
6. Yang, Y.H., et al. 2006. Antibodies against the activated coagulation Factor X (FXa) in the antiphospholipid syndrome that interfere with the FXa inactivation by antithrombin. *J. Immunol.* 177: 8219-8225.
7. Todd, T., et al. 2006. Severe Factor X deficiency due to a homozygous mutation (Cys364-Arg) that disrupts a disulphide bond in the catalytic domain. *Haemophilia* 12: 621-624.

## CHROMOSOMAL LOCATION

Genetic locus: F10 (human) mapping to 13q34.

## PRODUCT

Factor X 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Factor X shRNA Plasmid (h): sc-40405-SH and Factor X shRNA (h) Lentiviral Particles: sc-40405-V as alternate gene silencing products.

For independent verification of Factor X (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-40405A, sc-40405B and sc-40405C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

Factor X siRNA (h) is recommended for the inhibition of Factor X 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  $\mu$ M in 66  $\mu$ 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

Factor X (CaFX-50): sc-101370 is recommended as a control antibody for monitoring of Factor X 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Factor X gene expression knockdown using RT-PCR Primer: Factor X (h)-PR: sc-40405-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.