

Factor VIII siRNA (h): sc-43756

BACKGROUND

Factor VIII is a glycoprotein cofactor that serves as a critical component in the blood coagulation pathway. Insufficient expression levels or expression of nonfunctional Factor VIII results in hemophilia A, a common severe hereditary bleeding disorder. In the liver, the main site of factor VIII synthesis, the mature polypeptide chain of 2,332 amino acids is secreted into the lumen of the endoplasmic reticulum, where it interacts with various chaperone proteins, including Calreticulin, Calnexin and IgG-binding protein. From the lumen, a portion of Factor VIII translocates to the Golgi and undergoes activation via proteolysis of both the heavy and light chain portions of the protein into three fragments. Finally, proteolysis of activated Factor VIII by Factor Xa, Protein C or Thrombin results in inactivation of Factor VIII. Survival of Factor VIII in the bloodstream requires binding to von Willebrand factor (VWF) at both the amino and carboxy termini of the light chain. Point mutations occurring in those binding domains as well as at other active sites of Factor VIII likely underly 90-95% of disease cases.

REFERENCES

1. Fulcher, C.A., et al. 1983. THrombin proteolysis of purified factor VIII: correlation of activation with generation of a specific polypeptide. *Blood* 61: 807-811.
2. Eaton, D., et al. 1986. Proteolytic processing of human factor VIII: correlation of specific cleavages by thrombin, factor Xa, and activated protein C with activation and inactivation of factor VIII coagulant activity. *Biochemistry* 25: 505-512.
3. Foster, P.A., et al. 1989. Factor VIII structure and function. *Blood Rev.* 3: 180-191.
4. Kaufman, R.J. 1992. Biological regulation of factor VIII activity. *Annu. Rev. Med.* 43: 325-339.
5. Saenko, E.L., et al. 1997. The acidic region of the factor VIII light chain and the C2 domain together form the high affinity binding site for von willebrand factor. *J. Biol. Chem.* 272: 18007-18014.
6. Bhopale, G.M., et al. 2003. Blood coagulation factor VIII: an overview. *J. Biosci.* 28: 783-789.

CHROMOSOMAL LOCATION

Genetic locus: F8 (human) mapping to Xq28.

PRODUCT

Factor VIII 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 Factor VIII shRNA Plasmid (h): sc-43756-SH and Factor VIII shRNA (h) Lentiviral Particles: sc-43756-V as alternate gene silencing products.

For independent verification of Factor VIII (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-43756A, sc-43756B and sc-43756C.

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

Factor VIII siRNA (h) is recommended for the inhibition of Factor VIII 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

Factor VIII light chain (RFFVIII C/5): sc-59512 is recommended as a control antibody for monitoring of Factor VIII 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 MarkerTM 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 Factor VIII gene expression knockdown using RT-PCR Primer: Factor VIII (h)-PR: sc-43756-PR (20 μ 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 for detailed protocols and support products.