

VWF siRNA (r): sc-270212

BACKGROUND

Von Willebrand disease is a congenital bleeding disorder caused by defects in the von Willebrand factor protein (VWF). VWF is a multimeric glycoprotein that is found in endothelial cells, plasma and platelets, and it is involved in the coagulation of blood at injury sites. VWF acts as a carrier protein for Factor VIII, a cofactor required for coagulation, and it promotes platelet adhesion and aggregation. Several factors are known to stimulate the binding of VWF to platelets, including glycoprotein 1 β , ristocetin, botrocetin, collagen, sulphatides and heparin. Of the several domains contained within VWF, the A1, A2 and A3 domains have been shown to mediate this activation. VWF is thought to undergo a variety of posttranslational modifications that influence the affinity and availability for Factor VIII, including cleavage of the propeptide and formation of N-terminal intersubunit disulfide bonds.

REFERENCES

1. Naiem, M., et al. 1982. The value of immunohistological screening in the production of monoclonal antibodies. *J. Immunol. Methods* 50: 145-160.
2. Wise, R.J., et al. 1991. The role of von Willebrand factor multimers and propeptide cleavage in binding and stabilization of Factor VIII. *J. Biol. Chem.* 266: 21948-21955.
3. Fischer, B.E., et al. 1996. Effect of multimerization of human and recombinant von Willebrand factor on platelet aggregation, binding to collagen and binding of coagulation Factor VIII. *Thromb. Res.* 84: 55-66.
4. Ward, C.M., et al. 1997. Binding of the von Willebrand factor A1 domain to histone. *Thromb. Res.* 86: 469-477.
5. Jenkins, P.V., et al. 1998. Molecular modeling of ligand and mutation sites of the type A domains of human von Willebrand factor and their relevance to von Willebrand's disease. *Blood* 91: 2032-2044.
6. Bendetowicz, A.V., et al. 1998. Binding of Factor VIII to von Willebrand factor is enabled by cleavage of the von Willebrand factor propeptide and enhanced by formation of disulfide-linked multimers. *Blood* 92: 529-538.
7. Mazurier, C., et al. 1998. Molecular genetics of von Willebrand disease. *Ann. Genet.* 41: 34-43.

CHROMOSOMAL LOCATION

Genetic locus: *Vwf* (rat) mapping to 4q42.

PRODUCT

VWF siRNA (r) 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 VWF shRNA Plasmid (r): sc-270212-SH and VWF shRNA (r) Lentiviral Particles: sc-270212-V as alternate gene silencing products.

For independent verification of VWF (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270212A, sc-270212B and sc-270212C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

VWF siRNA (r) is recommended for the inhibition of VWF expression in rat 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

VWF (C-12): sc-365712 is recommended as a control antibody for monitoring of VWF 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 VWF gene expression knockdown using RT-PCR Primer: VWF (r)-PR: sc-270212-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.