SANTA CRUZ BIOTECHNOLOGY, INC.

GPVI siRNA (h): sc-43813

BACKGROUND

CD32 (also designated Fc γ RII) is a low affinity receptor for the Fc fragment of aggregated IgG. CD32 is responsible for the clearance of immunocomplexes by macrophages and also plays an important role in the regulation of antibody production by B cells. A member of the immunoglobulin superfamily, glycoprotein VI (GPVI), is a collagen receptor that plays a critical role in collagen-induced platelet aggregation. Patients who are deficient in GPVI suffer from bleeding disorders, and GPVI may be involved with cardiovascular and cerebral vascular diseases. GPVI also binds the collagen related peptide (CRP) and convulxin (Cvx), a GPVI-specific ligand from snake venom. GPVI mediates its signal through CD32, which in response to Cvx, leads to tyrosine phosphorylation and activation of Syk and PLCγ2. The gene encoding human GPVI maps to chromosome 19q13.42 and produces three isoforms, full length GPVI-1 and two additional isoforms, GPVI-2 and GPVI-3.

REFERENCES

1. Barclay, A.N., et al. 1993. The Leukocyte Antigen Facts Book. London. Academic Press, 170-172.
2. Sondermann, P., et al. 1999. Characterization and crystallization of soluble human Fc γ receptor II (CD32) isoforms produced in insect cells. Biochemistry 38: 8469-8477.
3. Jandrot-Perrus, M., et al. 2000. Cloning, characterization, and functional studies of human and mouse glycoprotein VI: a platelet-specific collagen receptor from the immunoglobulin superfamily. Blood 96: 1798-1807.
4. Asazuma, N., et al. 2000. Interaction of linker for activation of T cells with multiple adapter proteins in platelets activated by the glycoprotein VI-selective ligand, convulxin. J. Biol. Chem. 275: 33427-33434.
5. Ezumi, Y., et al. 2000. Molecular cloning, genomic structure, chromosomal localization, and alternative splice forms of the platelet collagen receptor glycoprotein VI. Biochem. Biophys. Res. Commun. 277: 27-36.
6. Lagrue-Lak-Hal, A.H., et al. 2001. Expression and function of the collagen receptor GPVI during megakaryocyte maturation. J. Biol. Chem. 276: 15316-15325.
7. Nieswandt, B., et al. 2001. Glycoprotein VI but not α2β1 integrin is essential for platelet interaction with collagen. EMBO J. 20: 2120-2130.

CHROMOSOMAL LOCATION

Genetic locus: GP6 (human) mapping to 19q13.42.

PRODUCT

GPVI 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GPVI shRNA Plasmid (h): sc-43813-SH and GPVI shRNA (h) Lentiviral Particles: sc-43813-V as alternate gene silencing products.

For independent verification of GPVI (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-43813A, sc-43813B and sc-43813C.

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

GPVI siRNA (h) is recommended for the inhibition of GPVI 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 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

GPVI (H-5): sc-390410 is recommended as a control antibody for monitoring of GPVI 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 GPVI gene expression knockdown using RT-PCR Primer: GPVI (h)-PR: sc-43813-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.