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RhAG siRNA (m): sc-152843

BACKGROUND

The Rh proteins in the red blood cell form a complex made up of one D-subunit, one CE-subunit and two Rh-associated glycoprotein (RhAG) subunits. Along with its antigenic properties, this complex functions as a major interaction site between the membrane lipid bilayer and the cytoskeleton of the red cell, via Ankyrin R interaction with the C-terminal cytoplasmic domain of the Rh and RhAG proteins. Furthermore, studies comparing ammonium concentration in normal and Rh(null) red cells show that the complex also contributes to ammonium export from the cells. Rh(null) is a rare autosomal recessive disorder characterized by an absence of Rh antigens and a varying degree of hemolytic anemia and spherostomatocytosis. The associated genetic mutations effect the transmembrane domain of the protein, correlating the structural defect with the loss of transport function characteristic in these cells.

REFERENCES

- Huang, C.H., et al. 1999. Molecular basis for Rh(null) syndrome: identification of three new missense mutations in the Rh50 glycoprotein gene. *Am. J. Hematol.* 62: 25-32.
- Suyama, K., et al. 2000. Surface expression of Rh-associated glycoprotein (RhAG) in nonerythroid COS-1 cells. *Blood* 95: 336-341.
- Mouro-Chanteloup, I., et al. 2002. Cell-surface expression of RhD blood group polypeptide is posttranscriptionally regulated by the RhAG glycoprotein. *Blood* 100: 1038-1047.
- Nicolas, V., et al. 2003. Rh-RhAG/Ankyrin R, a new interaction site between the membrane bilayer and the red cell skeleton, is impaired by Rh(null)-associated mutation. *J. Biol. Chem.* 278: 25526-25533.
- Hemker, M.B., et al. 2003. The Rh complex exports ammonium from human red blood cells. *Br. J. Haematol.* 122: 333-340.

CHROMOSOMAL LOCATION

Genetic locus: Rhag (mouse) mapping to 17 B2.

PRODUCT

RhAG siRNA (m) 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 RhAG shRNA Plasmid (m): sc-152843-SH and RhAG shRNA (m) Lentiviral Particles: sc-152843-V as alternate gene silencing products.

For independent verification of RhAG (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-152843A, sc-152843B and sc-152843C.

PROTOCOLS

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

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

RhAG siRNA (m) is recommended for the inhibition of RhAG expression in mouse 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

RhAG (D-5): sc-390045 is recommended as a control antibody for monitoring of RhAG 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 RhAG gene expression knockdown using RT-PCR Primer: RhAG (m)-PR: sc-152843-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.