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spectrin α I shRNA (h) Lentiviral Particles: sc-43430-V

BACKGROUND

Spectrin, an Actin binding protein that is a major component of the cytoskeletal superstructure of the erythrocyte plasma membrane, is essential in determining the properties of the membrane including its shape and deformability. Spectrins function as membrane organizers and stabilizers, composed of non-homologous α and β chains, which aggregate side-to-side in an antiparallel fashion to form dimers, tetramers and higher polymers. Spectrin α I and spectrin β I are present in erythrocytes, whereas spectrin α II (also designated fodrin α) and spectrin β II (also designated fodrin β) are present in other somatic cells. The spectrin tetramers in erythrocytes act as barriers to lateral diffusion, but spectrin dimers seem to lack this function. Defects of spectrin α I are present in the erythrocytes of many patients with abnormalities of red blood cell shape including hereditary spherocytosis and elliptocytosis.

REFERENCES

1. Speicher, D.W. 1986. The present status of erythrocyte spectrin structure: the 106-residue repetitive structure is a basic feature of an entire class of proteins. *J. Cell. Biochem.* 30: 245-258.
2. Gardner, K., et al. 1987. Modulation of spectrin-actin assembly by erythrocyte adducin. *Nature* 328: 359-362.
3. Coleman, T.R., et al. 1989. Functional diversity among spectrin isoforms. *Cell Motil. Cytoskeleton* 12: 225-247.
4. Saxton, M.J. 1989. The spectrin network as a barrier to lateral diffusion in erythrocytes. A percolation analysis. *Biophys. J.* 55: 21-28.
5. Kennedy, S.P., et al. 1994. A partial structural repeat forms the heterodimer self-association site of all β -spectrins. *J. Biol. Chem.* 269: 11400-11408.

CHROMOSOMAL LOCATION

Genetic locus: SPTA1 (human) mapping to 1q23.1.

PRODUCT

spectrin α I shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see spectrin α I siRNA (h): sc-43430 and spectrin α I shRNA Plasmid (h): sc-43430-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

spectrin α I shRNA (h) Lentiviral Particles is recommended for the inhibition of spectrin α I expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

spectrin α I (B-12): sc-271130 is recommended as a control antibody for monitoring of spectrin α I 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor spectrin α I gene expression knockdown using RT-PCR Primer: spectrin α I (h)-PR: sc-43430-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.