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Ankyrin-1 shRNA (m) Lentiviral Particles: sc-43262-V

BACKGROUND

Members of the Ankyrin family of proteins mediate the attachment of integral membrane proteins to the cytoskeleton. ANK1, ANK2 and ANK3 genes encode for the proteins in this family, Ankyrin-1 (also designated Ankyrin R), Ankyrin B and Ankyrin G, respectively. The proteins are structured similarly each composed of an N-terminal domain with multiple ankyrin repeats, a highly conserved central spectrin binding domain, and C-terminal regulatory domains which are susceptible to the most variance. Ankyrin-1 is a membrane protein that links the cytoskeleton to the plasma membrane in erythrocytes, cardiac and skeletal muscle, and brain. It is expressed as many isoforms, including a full length protein and several shorter isoforms. Ankyrin-1 has also been found to be defective in patients with hereditary spherocytosis (HS), a common hemolytic anemia.

REFERENCES

1. Eber, S.W., et al. 1996. Ankyrin-1 mutations are a major cause of dominant and recessive hereditary spherocytosis. *Nat. Genet.* 13: 214-218.
2. Zhou, D., et al. 1997. Small, membrane-bound, alternatively spliced forms of Ankyrin-1 associated with the sarcoplasmic reticulum of mammalian skeletal muscle. *J. Cell Biol.* 136: 621-631.
3. Gallagher, P.G., et al. 1997. Structure and organization of the human Ankyrin-1 gene. Basis for complexity of pre-mRNA processing. *J. Biol. Chem.* 272: 19220-19228.
4. Zhang, X., et al. 1998. Restriction of 480/270-kD ankyrin G to axon proximal segments requires multiple ankyrin G-specific domains. *J. Cell Biol.* 142: 1571-1581.

CHROMOSOMAL LOCATION

Genetic locus: Ank1 (mouse) mapping to 8 A2.

PRODUCT

Ankyrin-1 shRNA (m) 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 µl 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 Ankyrin-1 siRNA (m): sc-43262 and Ankyrin-1 shRNA Plasmid (m): sc-43262-SH as alternate gene silencing products.

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.

APPLICATIONS

Ankyrin-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of Ankyrin-1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

Ankyrin-1 (K-20): sc-87551 is recommended as a control antibody for monitoring of Ankyrin-1 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Ankyrin-1 gene expression knockdown using RT-PCR Primer: Ankyrin-1 (m)-PR: sc-43262-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.