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α-Dystrobrevin shRNA (m) Lentiviral Particles: sc-43322-V

BACKGROUND

Dystrobrevins are protein components of the dystrophin complex, whose disruption leads to Duchenne muscular dystrophy and related diseases. α-Dystrobrevin is a dystrophin-related and -associated protein that is involved in synapse maturation and is required for normal muscle function. α-Dystrobrevin is a component of the dystrophin glycoprotein complex. It is localized to the cytoplasmic side of the sarcolemma and is highly concentrated at the neuromuscular junctions in skeletal muscle. The insertion of 57 amino acids by alternative splicing accounts for the increase in molecular mass of α-Dystrobrevin 1 in skeletal and cardiac muscle compared with brain and lung. α-Dystrobrevin containing complexes are found in endothelial and smooth muscle cells, while β-Dystrobrevin containing complexes are present at the basal region of renal epithelial cells. Additionally, β-Dystrobrevin is found in neurons and is highly enriched in postsynaptic densities. Alternative splicing of α-Dystrobrevin produces γ-Dystrobrevin (isoform 5), δ-Dystrobrevin (isoform 7), ε-Dystrobrevin (isoform 6) and ζ-Dystrobrevin (isoform 8). Additional isoforms may also exist.

REFERENCES

1. Blake DJ, et al. 1998. β-Dystrobrevin, a member of the dystrophin-related protein family. *Proc. Natl. Acad. Sci. USA*. 95: 241-246.
2. Blake, D.J., et al. 1999. Different dystrophin-like complexes are expressed in neurons and glia. *J. Cell Biol.* 147: 645-658.
3. Loh, N.Y., et al. 2000. Assembly of multiple Dystrobrevin-containing complexes in the kidney. *J. Cell Sci.* 113: 2715-2724.
4. Gieseler, K., et al. 2001. Molecular, Genetic and Physiological Characterization of Dystrobrevin-like (dyb-1) Mutants of *Caenorhabditis elegans*. *J. Mol. Biol.* 307: 107-117.

CHROMOSOMAL LOCATION

Genetic locus: Dtna (mouse) mapping to 18 A2.

PRODUCT

α-Dystrobrevin 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 x 10⁶ 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 α-Dystrobrevin siRNA (m): sc-43322 and α-Dystrobrevin shRNA Plasmid (m): sc-43322-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

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0 x 10⁶ 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

α-Dystrobrevin (F-6): sc-376782 is recommended as a control antibody for monitoring of α-Dystrobrevin 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 α-Dystrobrevin gene expression knockdown using RT-PCR Primer: α-Dystrobrevin (m)-PR: sc-43322-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.

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.