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utrophin shRNA (h) Lentiviral Particles: sc-43494-V

BACKGROUND

Dystrophin and utrophin are related structural, Actin-binding proteins that are involved in anchoring the cytoskeleton to the plasma membrane. Dystrophin is the protein product of the Duchenne/Becker muscular dystrophy gene. Dystrophin expression is found in muscle and brain tissues, where it is localized to the inner surface of the plasma membrane. It has been speculated that alternative splicing of the carboxy terminus allows dystrophin to interact with a variety of proteins. Research has shown that the loss of dystrophin-associated proteins in Duchenne afflicted muscle is due to the absence of dystrophin rather than to muscle degradation and that the lack of dystrophin results in the loss of linkage between the cytoskeleton and the extracellular matrix. Evidence suggests that the upregulation of utrophin can reduce the dystrophic pathology.

REFERENCES

1. Monaco, A.P. 1989. Dystrophin, the protein product of the Duchenne/Becker muscular dystrophy gene. *Trends Biochem. Sci.* 14: 412-415.
2. Voit, T., et al. 1991. Dystrophin as a diagnostic marker in Duchenne and Becker muscular dystrophy. Correlation of immunofluorescence and western blot. *Neuropediatrics* 22: 152-162.
3. Ervasti, J.M., et al. 1993. Dystrophin-associated glycoproteins: their possible roles in the pathogenesis of Duchenne muscular dystrophy. *Mol. Cell Biol. Hum. Dis. Ser.* 3: 139-166.
4. Suzuki, A., et al. 1994. Molecular organization at the glycoprotein-complex-binding site of dystrophin. Three dystrophin-associated proteins bind directly to the carboxy-terminal portion of dystrophin. *Eur. J. Biochem.* 220: 283-292.

CHROMOSOMAL LOCATION

Genetic locus: UTRN (human) mapping to 6q24.2.

PRODUCT

utrophin 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 utrophin siRNA (h): sc-43494 and utrophin shRNA Plasmid (h): sc-43494-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

utrophin shRNA (h) Lentiviral Particles is recommended for the inhibition of utrophin 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

utrophin (A-7): sc-365955 is recommended as a control antibody for monitoring of utrophin 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 utrophin gene expression knockdown using RT-PCR Primer: utrophin (h)-PR: sc-43494-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.