SANTA CRUZ BIOTECHNOLOGY, INC.

utrophin siRNA (m): sc-43495

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.
5. Winder, S.J., et al. 1995. Utrophin actin binding domain: analysis of Actin binding and cellular targeting. J. Cell Sci. 108: 63-71.
6. Tinsley, J.M., et al. 1996. Amelioration of the dystrophic phenotype of mdx mice using a truncated utrophin transgene. Nature 384: 349-353.
7. Rybakova, I.N., et al. 1996. A new model for the interaction of dystrophin with F-Actin. J. Cell Biol. 135: 661-672.

CHROMOSOMAL LOCATION

Genetic locus: Utrn (mouse) mapping to 10 A1.

PRODUCT

utrophin 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see utrophin shRNA Plasmid (m): sc-43495-SH and utrophin shRNA (m) Lentiviral Particles: sc-43495-V as alternate gene silencing products.

For independent verification of utrophin (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-43495A, sc-43495B and sc-43495C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

utrophin siRNA (m) is recommended for the inhibition of utrophin 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 µM in 66 µl. 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

utrophin (8A4): sc-33700 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 utrophin gene expression knockdown using RT-PCR Primer: utrophin (m)-PR: sc-43495-PR (20 µl). 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.

PROTOCOLS

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