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LARGE shRNA (m) Lentiviral Particles: sc-44966-V

SANTA CRUZ®

BIOTECHNOLOGY

The Power to Question

BACKGROUND

Glycosyltransferase-like protein LARGE, also designated acetylglucosaminyl-transferase-like protein, belongs to the glycosyltransferase 8 family. This ubiquitously expressed protein is a type II membrane protein. Although it is widely expressed, highest levels of detection are in heart, brain and skeletal muscle. LARGE carries out the synthesis of glycosphingolipid and glycoprotein sugar chains and is part of the repeated disaccharide unit addition. It may also be important in the hyperglycosylation of α-dystroglycan. This interaction of LARGE with dystroglycan is crucial for the biosynthetic pathway to create functional dystroglycan. Loss of functional dystroglycan can result in muscle degeneration. The gene encoding for LARGE maps to chromosome 22q12.3, and defects in this gene can cause congenital muscular dystrophy, an autosomal recessive disorder. LARGE co-localizes with GM130, a Golgi marker.

REFERENCES

1. Grewal, P.K., et al. 2001. Mutant glycosyltransferase and altered glycosylation of α-dystroglycan in the myodystrophy mouse. Nat. Genet. 28: 151-154.

2. Holzfeind, P.J., et al. 2002. Skeletal, cardiac and tongue muscle pathology, defective retinal transmission, and neuronal migration defects in the LARGE (myd) mouse defines a natural model for glycosylation-deficient muscle-eye-brain disorders. Hum. Mol. Genet. 11: 2673-2687.

3. Barresi, R., et al. 2004. LARGE can functionally bypass α-dystroglycan glycosylation defects in distinct congenital muscular dystrophies. Nat. Med. 10: 696-703.

4. Kanagawa, M., et al. 2004. Molecular recognition by LARGE is essential for expression of functional dystroglycan. Cell 117: 953-964.

5. Brockington, M., et al. 2005. Localization and functional analysis of the LARGE family of glycosyltransferases: significance for muscular dystrophy. Hum. Mol. Genet. 14: 657-665.

CHROMOSOMAL LOCATION

Genetic locus: Large (mouse) mapping to 8 B3.3.

PRODUCT

LARGE 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 LARGE siRNA (m): sc-44966 and LARGE shRNA Plasmid (m): sc-44966-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

LARGE shRNA (m) Lentiviral Particles is recommended for the inhibition of LARGE 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

LARGE (Y-14): sc-33435 is recommended as a control antibody for monitoring of LARGE 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor LARGE gene expression knockdown using RT-PCR Primer: LARGE (m)-PR: sc-44966-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.