Produktinformation

🔵 Forschungsprodukte & Biochemikalien

🔵 Zellkultur & Verbrauchsmaterial

🔵 Diagnostik & molekulare Diagnostik

🔵 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

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MuSK shRNA (m) Lentiviral Particles: sc-44655-V

BACKGROUND

Receptor tyrosine kinases (RTKs) represent an important class of transmembrane signaling molecules. Binding of the extracellular domain of an RTK to its cognate ligand leads to receptor dimerization and the activation of the intrinsic tyrosine kinase activity of its intracellular kinase domain. The specificity of this type of cellular communication is conferred in part by the distribution of the receptor, which determines the cells that are capable of responding to a given ligand. MuSK, for muscle-specific kinase, is an RTK that is uniquely specific to the skeletal muscle lineage. MuSK is expressed at low levels in proliferating myoblasts, but is induced upon terminal differentiation and myotube fusion. In the embryo, MuSK is expressed in developing muscle, but its level of expression is dramatically reduced in mature muscle, where it is abundant only at the neuromuscular junction. The human MuSK gene maps to chromosome 9q31.3, overlapping a region containing the Fukuyama muscular dystrophy mutation.

REFERENCES

1. Shoyab, M., et al. 1989. Structure and function of human amphiregulin: a member of the epidermal growth factor family. Science 243: 1074-1076.

2. Janssen, J.W.G., et al. 1991. A novel putative tyrosine kinase receptor with oncogenic potential. Oncogene 6: 2113-2120.

3. Schlessinger, J., et al. 1992. Growth factor signaling by receptor tyrosine kinases. Neuron 9: 383-391.

4. Biesecker, L.G., et al. 1995. Identification of alternative exons, including a novel exon, in the tyrosine kinase receptor gene Etk2/tyro3 that explain differences in 5' cDNA sequences. Oncogene 10: 2239-2242.

5. Taylor, I.C.A., et al. 1995. Overexpression of the Sky receptor tyrosine kinase at the cell surface or in the cytoplasm results in ligand-independent activation. Oncogene 11: 2619-2626.

6. Valenzuela, D.M., et al. 1995. Receptor tyrosine kinase specific for the skeletal muscle lineage: expression in embryonic muscle, at the neuromuscular junction, and after injury. Neuron 15: 573-584.

CHROMOSOMAL LOCATION

Genetic locus: Musk (mouse) mapping to 4 B3.

PRODUCT

MuSK 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 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 MuSK siRNA (m): sc-44655 and MuSK shRNA Plasmid (m): sc-44655-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80º C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4º C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

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

SUPPORT REAGENTS

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

MuSK (C-19): sc-6009 is recommended as a control antibody for monitoring of MuSK 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 MuSK gene expression knockdown using RT-PCR Primer: MuSK (m)-PR: sc-44655-PR (20 µl, 515 bp). 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.

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