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MuRF1 shRNA (h) Lentiviral Particles: sc-43951-V

BACKGROUND

Muscle specific RING finger protein (MuRF1) is a sarcomere-associated protein that is upregulated by conditions that provoke atrophy. Pharmacological or genetic inhibition of the IKK β /NF κ B/MuRF1 pathway reverses muscle atrophy, which presents MuRF as a target for clinical intervention. MuRF1 is a key regulator of the PKC-dependent hypertrophic response and can blunt cardiomyocyte hypertrophy, which may have important implications in the pathophysiology of clinical cardiac hypertrophy. MuRF1 directly associates with Titin kinase and influences microtubule-dependent signaling pathways in striated muscle and iris. MuRF1 upregulation is an indicator for skeletal muscle atrophy mechanisms that utilize ubiquitin-dependent proteolysis. MuRF1 transcript levels are high in situations where there is an overabundance of reactive oxygen species, such as cancer, AIDS and sepsis.

REFERENCES

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- Glass, D.J. 2003. Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat. Cell Biol.* 5: 87-90.
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- Sachek, J.M., et al. 2004. IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogen-1 and MuRF1. *Am. J. Physiol. Endocrinol. Metab.* 287: E591-E601.

CHROMOSOMAL LOCATION

Genetic locus: TRIM63 (human) mapping to 1p36.11.

PRODUCT

MuRF1 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 MuRF1 siRNA (h): sc-43951 and MuRF1 shRNA Plasmid (h): sc-43951-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.

APPLICATIONS

MuRF1 shRNA (h) Lentiviral Particles is recommended for the inhibition of MuRF1 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

MuRF1 (C-11): sc-398608 is recommended as a control antibody for monitoring of MuRF1 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 MuRF1 gene expression knockdown using RT-PCR Primer: MuRF1 (h)-PR: sc-43951-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.

PROTOCOLS

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