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MAFbx shRNA (h) Lentiviral Particles: sc-43906-V

BACKGROUND

Muscle atrophy F-box (MAFbx) is an E3 ubiquitin ligase that initiates ATP-dependent ubiquitin-mediated proteolysis and promotes muscle atrophy. MAFbx transcript is abundant in cardiac and skeletal muscle undergoing atrophy. MAFbx^{-/-} mice are resistant to muscle atrophy. MAFbx is thought to recognize and bind to some phosphorylated proteins and promote their ubiquitination and degradation during skeletal muscle atrophy. It interacts with MyoD by ubiquitination via a sequence found in transcriptional coactivators and therefore may play an important role in the course of muscle differentiation by determining the abundance of MyoD. MAFbx is specifically expressed in cardiac and skeletal muscle.

REFERENCES

1. Bodine, S.C., et al. 2001. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294: 1704-1708.
2. Dehoux, M.J., et al. 2003. Induction of MafBx and Murf ubiquitin ligase mRNAs in rat skeletal muscle after LPS injection. *FEBS Lett.* 544: 214-217.
3. Glass, D.J. 2003. Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat. Cell. Biol.* 5: 87-90.
4. Dehoux, M., et al. 2004. Role of the Insulin-like growth factor I decline in the induction of atrogen-1/MAFbx during fasting and diabetes. *Endocrinology* 145 :4806-4812.
5. Rourke, B.C., et al. 2004. Myosin isoform expression and MAFbx mRNA levels in hibernating golden-mantled ground squirrels (*Spermophilus lateralis*). *Physiol. Biochem. Zool.* 77: 582-593.
6. Tintignac, L.A., et al. 2005. Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase. *J. Biol. Chem.* 280: 2847-2856.

CHROMOSOMAL LOCATION

Genetic locus: FBX032 (human) mapping to 8q24.13.

PRODUCT

MAFbx 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 MAFbx siRNA (h): sc-43906 and MAFbx shRNA Plasmid (h): sc-43906-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

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

MAFbx (L-15): sc-27645 is recommended as a control antibody for monitoring of MAFbx 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 MAFbx gene expression knockdown using RT-PCR Primer: MAFbx (h)-PR: sc-43906-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.